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Unusually versatile plant genus *Azadirachta* with many useful and so far incompletely exploited properties for agriculture, medicine and industry

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Azadirachta indica (Indian Neem), *A. siamensis* (Thai Neem) and *A. excelsa* (Marrango, Philippinean/Malaysian Neem) belong to the Mahogany family and are endemic to the Indian subcontinent, with subsequent anthropogenic expansion to South-East Asia, but also into all tropical and subtropical areas of the globe. Traditional neem uses in stored product protection and medicine in India are well established for thousands of years. Recently, modern methods of analytical, organic and biochemistry could explain biochemical mechanisms of action. This was followed by greatly expanded insect management practices based on the useful properties of neem and neem oil. These natural products can serve as insect phago-deterrents and fitness reducing agents against pest insects, but also against bacterial, fungal and virus diseases of plants, domestic animals and humans. Neem products, in concert with pheromones, are the general agents of choice if all other control measures for various reasons fail.

Keywords: *Azadirachta spp.*, azadirachtin, IPM, marrango, neem, neem oil

1 Introduction

Organic agriculture is in substantial need of natural, non-toxic and -ecotoxic, sustainable and renewable agents for pest arthropod management. Since synthetic toxins do not longer convincingly and responsibly qualify for this immense tasks, only few agents with novel mechanisms of action are available at all. Novel mechanisms sought are behavioural modifiers (as in pheromones, kairomones and allomones which can artificially attract, or repel), agents with fitness reducing properties from plants and from a limited number of organisms within the microbial and aquatic scene.

This contribution provides information on a few novel key uses of neem in agriculture, human and veterinary medicine, both from our own research and from the meanwhile abundant scientific literature with its about 5000 entries (CAB Abstracts search via OVID).

Today, neem is cultivated in all tropical and subtropical regions of the globe. Numerous agricultural, sociocultural, veterinary medical, medical, and industrial uses have been described (Eppler et al. 1986; Schmutterer et al. 1981, 2002; Schmutterer und Ascher 1984, 1987; Schmutterer 1995, 2002A/B, 2005; Ahmed 2002; Ketkar & Ketkar 2002; Rembold & Mwangi 2002; Schmutterer & Singh 2002; Talwar et al. 2002; Schmutterer and Huber 2005; Van der Esch et al. 2009). Major parts of the history and use of neem have been dealt with by several authors (Butterworth & Morgan 1968; Bilton et al. 1987; Isman et al. 1996; Isman 1997, 2002; Ketkar 2009; Kraus 2002A/B; Koul 2004; Ley et al. 1993; Morgan & Allan 2002;

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Morgan 2006, 2009; National Research Council 1992; Rembold 2002; Saxena 2002A-D; Siddiqui et al. 2009; Veitch et al. 2007, 2008), and their respective co-workers, cited in Schmutterer (2002A). For a short review see also Hummel et al. (2012), for a major review see Morgan (2009). Instrumental analysis succeeded in characterizing and chemically identifying followed by synthesis of the major neem components azadirachtin, marrangin, salannin, nimbin and the ever expanding list of their congeners. Neem products are without toxicity for warm-blooded vertebrates and do little if any harm to pollinators and parasitoids at the third trophic level.

2 Material and Methods

Neem seeds (free from fungi in order to avoid contamination by fungal toxins of the aflatoxin type) can be processed for neem oil by churning and cold pressing. It can retain their natural (10-100 ppm) azadirachtin content which is sufficient for many field applications. Also, neem seeds can be milled and extracted with apolar solvents for removal of fats and oils and can be subsequently extracted with methyl t-butyl ether to yield a fairly well enriched (up to 10 %) extract with azadirachtin (or marrangin from *A. excelsa*) which still contains azadirachtin isomers as well as salannin and nimbin.

A higher azadirachtin content (neem seed kernel extracts (NSKE)) of 10 % and more of azadirachtin is useful for comparative studies in finding out which insects are susceptible for neem extracts. Highly purified extracts with azadirachtin, respectively marrangin, content of 95 % and better are gained by CCC (counter current chromatographic) purification from NSKE (Hummel et al. 1997, Hein & Hummel 1998, Hein 1999) with subsequent solvent removal *in vacuo*. For scientific mechanistic studies of biochemistry, pure crystalline material is needed that can be obtained by repeated HPLC.

Care must be taken to keep the storage or reaction medium in the near neutral pH range. Otherwise, hydrolysis and molecular rearrangements will split off or scramble the numerous ester groups of azadirachtin with loss of bioactivity. Also, sensitivity against photolysis has been observed.

Azadirachtin is now also available synthetically (Veitch et al. 2007, 2008), but it belongs to the very highly prized natural products and will not be affordable to the pest manager). Leaf, bark, and root material of neem also contain useful natural products with so far incompletely investigated properties.

Figure 1 (A) *A. indica* tree, (B) leaves and flowers, and (C) fruits. (D) *A. excelsa* tree in Thailand

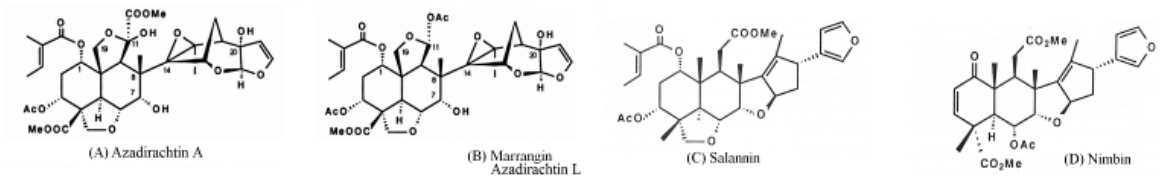

Figure 2 Major biologically active ingredients of the Indian neem (A), (C), and (D), namely Azadirachtin, Salannin and Nimbin, (B) Marrangin from Philippinean neem.

3 Results and Discussion

For many years, traditional Indian agriculture, stored product protection and medicine used *A. indica* leaves and neem fruit kernels (Fig. 1 A-C). Countries other than India like Thailand have access to *A. siamensis*, Malaysia and the Philippines to *A. excelsa*, each with specific compounds, many of whom are not completely characterized to date let alone structurally identified (for a review see Kraus 2002B). The leading structures in *A. indica* are azadirachtins A-K, salannin and nimbin (see fig. 2 A/C/D). Marrangin, occurring only in *A. excelsa*, is structurally very similar except around carbon centre 11 in ring C (Kalinowski et al. 1993). In some insect models it is biologically significantly more active than azadirachtin. Insect spp. studied are locusts, mites, Mexican bean beetles, and *Spodoptera* moths.

Synthetic efforts towards obtaining the aza structure have been successful (Ley et al. 1993, Veitch et al. 2007, 2008) but are more of academic than practical interest because of the lengthy and expensive reaction sequence required. More promising for continuously harvesting azadirachtin *in vitro* from suspension cultures of plant cells seemed to be the callus culture approach of Morgan and Allan (2002). Many obstacles including technical translation into manageable large scale production could, however, only incompletely be solved. Therefore, batch-wise solvent extraction of neem seeds to date continues to be the least expensive procedure for production of raw azadirachtin.

Neem products are formulated in a variety of ways. NSKE (Schmutterer et al. 1984, 2005; Schmutterer & Ascher 1987) and NeemAzal (Kleeberg 2001) are still the most widely used in Europe. The latter received official use permission by JKI Berlin, first for ornamentals, later for agricultural uses.

Most *general* information is contained in various monographs by Schmutterer (Schmutterer et al. 1981, 1984, Schmutterer & Ascher 1987) and two seminal books by Schmutterer (Schmutterer 1995, 2002A) but also in the volume of National Research Council (1992) and in Schmutterer (1988). Also Siddiqui et al. (2009) reported on exciting new chemistry in neem natural products. *Specific* information can be found in 14 volumes edited by H. Kleeberg and his associates. The last volume of this series was dedicated to biological control of plant, medical and veterinary pests by Strang and Kleeberg (2009).

To cite *specifics*, Saxena (2002D) covered many aspects of stored product protection. Schmutterer (2002A) wrote on household and fabric pests. Ketkar and Ketkar (2002) shed light on medicinal and pharmacological uses in Asia. Talwar et al. (2002) reported on fertility control and infections of the human reproductive tract. Landscape improving effects of neem received attention by Schmutterer (2002A).

Neem trees can serve as wind breaks and as soil erosion controls in Africa, where neem also serves as a premium shade tree. Due to low water requirements, it is used for greening of arid regions and deserts. Industrial uses are dealt with by Schmutterer (2002A), where neem oil, neem soap, neem wood, termite resistance of neem wood, high quality wood for furniture, neem cake as manure and nitrification inhibitor, neem seed oil for greases and cosmetics, oils and fuels for diesel engines are introduced (the latter after transesterification of neem oil

with methanol). General problems with registration of neem products as IPM agents are covered by Bode and Guske (2005) for Germany and for the US by Zubkoff and Kumar (1999), by Hellpap and Dreyer (2002) or by Hellpap et al. (2002), who deal with practical aspects of neem in IPM.

Biochemical studies by Mordue and Rembold of neem components confirm the notion that azadirachtin has multiple modes of action. This impedes and greatly delays the process of resistance acquisition in response to frequent neem applications. Interacting with the biochemical information transfer from RNA to proteins, corresponding the later transcriptional steps, azadirachtin interferes with protein synthesis (Mordue 2002, Rembold 2002). It will, in principle, modify expression of *all* enzymes responsible for food digestion in the insect gut and the processes at the gustatory level regulating food intake at the mouth. Indeed, neem works *primarily* as an antifeedant at the level of uptake and *secondarily*, if uptake has indeed occurred, as a motility reducing agent of the gut thus interfering with nutrient acquisition. *Thirdly* it works as a developmental modifier with effects on key tissues like egg and sperm producing cells. In addition, and *fourthly*, it interferes biochemically with ecdysterone and juvenile hormone metabolism and with morphogenesis, e.g. with the formation and function of imaginal discs which organize further differentiation of the immature to the mature insect body. Insects so treated will be partly unable to free themselves from their larval or pupal cuticle. Formation of crumpled wings with inability to fly is frequently seen in adult insects treated with neem at the larval stage. Moreover, higher sensory levels like responsiveness in receiving of or responding to mating signals are negatively affected in *Diabrotica* beetles exposed to maize plants treated with low levels of neem oil (Hummel 1989 A, B).

4 Conclusions

Neem is *not* the agent of choice for large scale, "industrial" agriculture as practiced in several parts of the world for cheap staple food production. Rather, its strength is in home and garden, in stored products protection, under glass culture, and in organic agriculture on smaller patches of farmland.

Remarkably, neem products are nontoxic to warm-blooded vertebrates. Due to their alternative modes of action, these products are much less prone to induce resistance than conventional chemical insecticides.

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