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Neem the tree of 21st century

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Abstract

During the last decay, the commercial importance of Neem tree (*Azadirachta indica* A. Juss) increased exponentially, as well as its global diffusion and the number of derived products in the market. Despite the identification of hundreds of constituents in the seed oil, the main marketed product, a lot of chemistry still must be carried on.

The high complexity of chemical composition and the derived multiple activity ask for an exceptional research validation. Azadiractins, among the main constituents of the oil and so far considered the responsible of the insecticide activity, could be only in part involved, calling for investigation on other constituents, including the degradation products.

Starting from reliable chemical data, Biology can take the center of the scene to validate neem as the multipurpose tree of the future. Starting from the actual medical and insecticidal applications it is time to explore other possible commercial applications in accordance with the future depicted by the International Research Institutions.

KEY WORDS: NEEM, AZADIRACHTA INDICA, AZADIRACTIN, INSECTICIDE

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Introduction

Natural substances are experiencing a Renaissance period. Prompted in the market by the general request of natural and the continuous appearance of new products, many fields are going to be totally changed by secondary products presence. Mainstream is crowded by a plethora of plant species, with important leaders. In 1989 WHO/UNEP considered Neem tree (*Azadirachta indica* A. Juss) one of the most promising tree of the 21st century evidencing its great potential in several fields and applications [1,2]. This hypothesis is fully confirmed. The importance of Neem increased exponentially during the last years. Considering the enormous quantity of results and scientific data concerning the validation of medicinal properties of Neem, the International Scientific Community [3] included Neem into the top ten list of plants to be investigated and used for the sustainable development of the planet and the health of living beings.

Actually a multitude of researchers and students are constantly at work to catch the secrets of Neem and every day this wonderful tree is able to give us any kind of surprise.

Ethnobotany

Neem pertains to the Meliaceae (Mahogany family). The genus *Azadirachta* consists of few species, wherein *A. indica* is by far the most important one for diffusion and applications [4-8]. Usually it is a beautiful, large, monumental, hardy tree. Indigenous to the Indo-Pak subcontinent (Pakistan, India, Bangladesh, Myanamar). It easily and rapidly grows, reaching 80 cm in one year. Owing to its ability of growing so easily and surviving on dry, nutrient-lean soil, it is now globally cultivated in tropical and subtropical countries, including South Asia, West Africa, central (Cuba) and South America, Australia, the Plains of Arafat. Actually it was introduced and heavily cultivated also in China. In some countries cultivars have been developed, like in Thailand, where the leaves of *A. indica* var. *siamensis* are used as vegetable and medicaments. Flowering occurs January to May.

Flowers are fragrant, beautiful and abundant. Fruits ripening June through August are green ellipsoidal drupes, containing one seed. A single mature tree may produce annually 5-8 Kg of seeds. However, it can be easily confused with another Meliacea, *Melia azedarach*, known as the Indian Lilac [9] for the colour of the abundant flowers; however, the two trees can be distinguished by the shape of the fruits.

Traditional uses

India is the original country of neem, where its commercial use is reported from the Vedic period over 4000 years B.C. [10]. The medicinal use of neem is strongly eradicated into the Indian tradition. All its parts are largely used for many illness and in Indian rural areas the plant is called "the village pharmacy".

The medicinal utilizations have been documented in the Atharva Veda, the Grihathyasutras and also in the Sutragranthas. Its medicinal properties are described in the 'Puranas' and neem is commonly used in 'Ayurvedic' and 'Unani' medicine to relieve so many different pains, fevers, infections and other complaints.

In Ayurvedic medicine system neem is used to treat malarial fevers [11]. Recent experiments have shown that one of neem's components, gedunin, is as effective as quinine against malaria. Malaria affects millions of people and is responsible for about 2 million deaths every year in India and several other countries.

Neem in agriculture, veterinary and environmental care

The Upavanavinod is an ancient Sanskrit treatise dealing with forestry, horticulture and agriculture; the neem finds prominence here as cure for ailing soils, plants and livestock [12-14]. Varahamihira's Brihat Samhita has a chapter of verses where neem is highly recommended, containing a description of

the trees that should be planted near one's house. Neem has also been traditionally used against various livestock insects such as maggots, horn flies, blowflies and biting flies, as well as for controlling some bacteria of veterinary importance and against intestinal worms in animals. Neem grows on most types of soils.

It can grow on compact clay or laterite crusts, however black cotton soils are preferred. Neem can persist even under very adverse soil conditions. It comes up well in moderately saline and alkali soils with high levels of sodium carbonate and bicarbonates. It thrives better than most other species on dry, stony shallow soils where vegetation is scarce. Therefore, if so far mainly attention was focused on pest management, its eco-friendly characters will favour its worldwide expansion by human aid, as actually occurring in several parts of Asia.

Chemistry

Chemistry of neem is very complicated and still far to be completed, despite the great number of dedicated researches. Hundreds of compounds have been isolated and identified from various parts of neem [15,16], with seeds as the most investigated for their commercial value. The seeds may contain approximately 45% of a brownish yellow oil, mainly constituted by several fatty acids, i.e. oleic (50-60%), palmitic (13-15%), stearic (14-19%), linoleic (8-16%) and arachidic (1-3%), although several other compositions have been reported.

Main character of the oil is the unpleasant strong alliaceous odour and the acrid taste, attributed to sulphurous constituents. However, the composition of neem's oil is high variable, depending from the origin of raw material, the extraction method, preservation and conservation.

Extraction can be executed with different temperature, pressure and methods, affecting the yield as well as the content. Differences in composition were also reported for the geographic origins and the collection seasons.

More than 300 compounds have been characterized from the seeds. One-third of them are nortriterpenoids, that means triterpenoid lacking of some carbon atoms. In many cases, partial loss of lateral chain is combined by a complicated rearranging of the remaining part is, giving rise to different polyclicic molecular skeletons full of oxygenated functional groups, partially acylated.

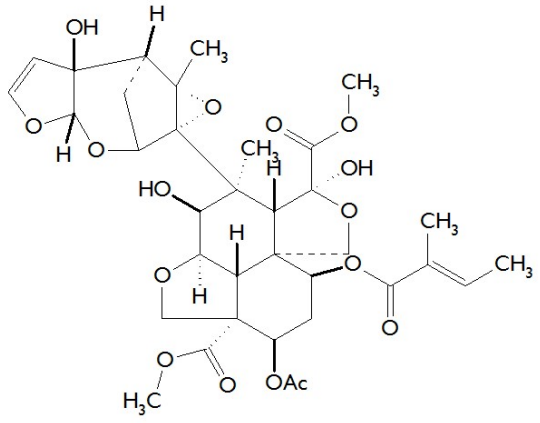
Nortriterpenoids are chemotaxomically well located in few related families of Rosidae Angiosperm Dicotyledons, i.e. Rutaceae, Simarubaceae, Cucurbitaceae, Meliaceae.

Nortriterpenes can be divided into two main groups: a) limonoids (C26) with partial loss of the lateral chain and quassinoids (C20 and C19) with total loss of the lateral chain and more. In ancient times plants containing these kind of compounds were mainly famous for bitterness of their drugs.

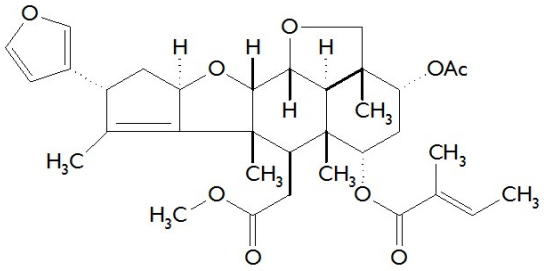
Later several other properties have been reported, including antioxidant, antimicrobial and antitumoral activities, beside the insecticidal of neem being so far the most important.

Neem limonoids belong to nine basic structures, with three main skeleton types: a) the azadirachtins, highly polioxygenated and acylated, with a saturated first ring, a tetrahydrofurane ring between the two first rings and a final dihydrofurane ring chained with the other part of the molecule; b) the nimbins, less oxygenated and acylated with a skeleton evidently similar that of the steroids, the furane ring with only a link with the remaining part of the molecule; c) the third type is similar to the azadirachtins one, but the complicated polycyclic part containing the dihydrofurane ring is less complicated, giving rise to a linear general skeleton. These differences are important either for the biological activity as for the decomposition.

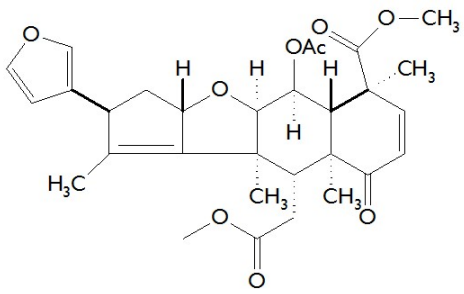
Neem's oil formulations usually show a range of different azadirachtins amounts, ranging from 1,000-4,000 mg/Kg, meaning that products can be obtained either by using poor neem oil or dilution process of neem extracts containing a quantity of azadirachtin, up to 5%.



azadirachtin A



salannin



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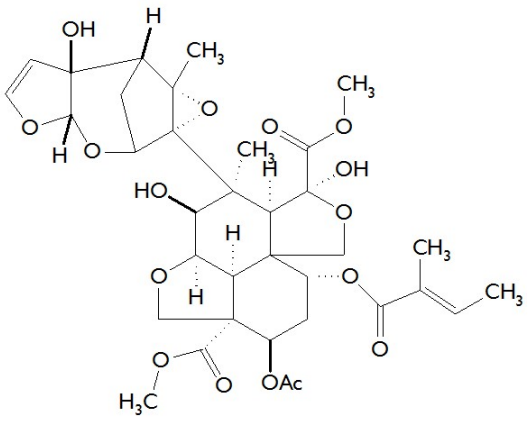

azadirachtin B

Neem in pest management

Neem has attracted worldwide attention for its insecticide activity, positively tested against 400 insect pests. Only for these reasons neem could be considered the tree with more expanding future in the 21st century, but its importance is related to the multipurpose utility. The insecticidal effects has been reported in several studies [17-25], including larvicidal induced by the neem oil, azadirachtin A and other nortriterpenes widely used in the manufacturing of insecticides.

Unlike chemical insecticides, neem compounds work on the insect's hormonal system, not on the digestive or nervous system and therefore does not lead to development of resistance in future generations. These compounds belong to a general class of natural products called 'limonoids'. The limonoids present in neem make it a harmless and effective insecticides, pesticide, nematicide, fungicide etc. The most significant limonoids found in neem with proven ability to block insect growth are: azadirachtin, salanin, meliantriol and nimbin.

Azadirachtin is currently considered as neem's main agent for controlling insects. It appears to cause 90% of the effect on most pests. It does not kill insects – at least not immediately –, instead both repels and disrupts their growth and reproduction. Research over the past years has shown that it is the most potent growth regulator and feeding deterrent ever assayed. It can repel or reduce the feeding of many species of pest insects as well as some nematodes. In fact, it is a so potent deterrent that a mere trace of its presence prevents some insects from even touching plants.

Control of pests in agriculture is a key problem for mankind future, on an average 18 % of the crop yield is lost due to pest damages. The pest control potential of neem in developing countries, however, remained largely untapped until DDT and other broad-spectrum synthetic insecticides were dominant. It is only in the past decade, that the pest control potential of neem, which does not kill pests but affects their behaviour and physiology, has been recognized. Neem's multiple effects are now

considered far more desirable than a quick knockdown in integrated pest management programs as they reduce the risk of environmental permanent damage and exposing pests natural enemies to poisoned food or starvation. Owing to neem's complicated chemistry, it acts in several ways, such as repellence, feeding and oviposition deterrence, growth inhibition, mating disruption, chemosterilization, etc.

Hormonal effects were the key of the inversion of strategy, changing the target from the adult everywhere dispersed to the locally maintained larvae. Certain hormones are necessary for the complicated growth and development of insects. These hormones control the process of metamorphosis as the insects pass from larva to pupa to adult.

Azadirachtin blocks those parts of the insect's brain that produce these vital hormones. When neem products, especially azadirachtin (aza), enter into the body of larvae, the activity of ecdysone enzyme is suppressed and the larva fails to molt, remains in larval stage and ultimately dies. If larva manages to enter the pupal stage due to low aza concentration it dies and still at low concentration of aza the adult emerging from the pupa is 100 % malformed, absolutely sterile without any capacity for reproduction. It is through these subtle hormonal effects that this important compound of neem breaks the life cycle of insects. The insect populations decline drastically as they become unable to reproduce. However, also antifeedant and deterrent activities are important to defend crops

Ecology

The ideal plant derived product, including insecticide, should be eco-friendly, sustainable, low cost and target specific leaving unaffected the beneficial ones. Actually, probably the most important character for an insecticide is biodegradability, that means an eco-friendly impact, being biodegradable by the action of sunlight, neem products do not leave any residue on the field. Clearly, this is a controversial aspect. if the degradation is too fast, the cost rises consequently, otherwise environmental accumulation could generate devastating collateral effect, as in the DDT case. Therefore, in comparison with synthetic insecticides, natural products derived are subjected to attack by several intrinsic and extrinsic factors, like temperature, exposure to radiations, pH, humidity generating hydrolyzes, racemisation, oxidation and therefore degradation. Neem's limonoids, considered the active constituents, are affected by photodecomposition. Experimental studies reported an half-time of azadirachtin in open space after dissipation of about 20 h. This short persistence is due to sunlighy exposure sensitive parts, like acylated sites, furan ring, epoxide and unsaturated linkages. The degradation can occur also when Neem products are stored under appropriate conditions. In this case the half-time was recorded in about 60 days. However the presence, or addition, of antioxidants such as ferulic acid, gallic acid and flavonoids is important in a degree of photostabilization [24].

These aspect are apparently eco-friendly, until the toxicity of degradation products of azadirachtins will be reported, but may generally discredit the utilization of Neem products. In any case, investigation on activity of other constituents, than azadirachtins could be useful. Neem products are relatively safe to mammals and humans [25,26]. The absence of toxicity is largely evidenced by the prominent role of fruits, bark, and leaves in Indian Traditional Medicine, as well as by the use of flowers and leaves as vegetables in Asian countries and the current utilization in a number of products in several parts of the market.

Neem cake, which looks more like flour than cake, is the residue that's left over when the kernel is crushed from neem seeds and remaining is pressed, to obtain the oil. The pressure can be made in different ways and at different temperature, affection the composition of the resulting product. Two products are therefore in the market: neem oil cake obtained by cold pressure, with 6% of the oil still residue, and neem cake deoiled, with a residue up to 1,5%. Actually it is not approved as pesticide and mainly it is highly appreciated as organic fertili-

zed. Neem cake has an adequate quantity of NPK in organic form for plant growth, but seems to make soil more fertile due to an ingredient that blocks soil bacteria from converting nitrogenous compounds into nitrogen gas. It prolongs the availability of nitrogen to both short duration and long duration crops, being much less concentrated than neem oil and therefore released more slowly. Neem cake acts as a natural fertilizer with pesticidal properties, protecting crops from nematodes, soil grubs and white ants.

It is compatible with soil microbes, improves and rhizosphere microflora. Neem is harmless to non-target and beneficial organisms like pollinators, honey bees, mammals and other vertebrates. The effect could be due to its residual limonoid content, but other constituents could be involved. Neem cake also reduce alkalinity in soil, as it produces organic acids on decomposition. and hence ensures fertility of the soil, improving the organic matter content of the soil and soil texture, water holding capacity, and soil aeration for better root development.

Also other parts are commercially utilized. The bark, as well as the oil, is used for cosmetics, health care products and medicinal preparations. The leaves are medicinal. The kernel of the neem fruit is crushed to yield an oil which is used chiefly in pest controlling preparations and also in medicinal, cosmetic and health care products. Even the twigs of the tree are used as toothbrushes for dental hygiene.

Future

India alone has an annual potential of 80,000 tons of oil and 330,000 tons of neem cake from 14 million plants that grow naturally. To this potentiality the high number of cultivations actually occurring in many parts of the world must be added. This situation evidences the neem's high sustainability and possibility to have an increasing production of low cost products to be utilized in many fields, not only insecticides, from medicine to the cosmetic one. The importance of the neem's future is strictly related to this wild range of utilizations, that are strictly linked to the new market of plant natural products. In this regard, applications in the antimicrobial activity are surely a promising field. The main relevant aspect of neem is its chemical complexity. The peculiar character of neem is the variability of the composition of its drugs and derived products. The case of neem cake is good example of the neem's potentialities to be explored. The spectacular amazing mirage is that there is always something of important to be discovered on this treasured gift of the Nature.

References

1. Nicoletti M, Maccioni O, Coccioletti T, Mariani S, Vitali F. Neem Tree (Azadirachta indica A. Juss) as source of Bioinsecticides. in Insecticides - Advances in Integrated Pest Management. Farzana Perveen (Ed.). Intech. Rijeka, Croatia, 2011; 411-428.
2. National Research Council (1992) Neem: a tree for solving global problems. Academic, Washington.
3. Rossi Forim M, da Silva MF das Gracas F, Fernandes JB. Secondary Metabolism as a Measurement of Efficacy of Botanical Extracts: The Use of Azadirachta indica (Neem) as a Model in Insecticides - Advances in Integrated Pest Management. Farzana Perveen (Ed.). Intech. Rijeka, Croatia. 2011, p. 367-390.
4. Singh RP, Saxena RC, Azadirachta indica A. Juss, Oxford 7 IBH Publishing Co. Pvt. Ltd., 1999
5. Schmutterer H, The Neem Tree, VCH. Verlagsgesellschaft mnH, Germany, 1995
6. Tewari DN, Monograph of Neem, Dehradun, International Book Distributors, India. 1992
7. Randhawa NS, Parmar BS eds Neem Research and Development, Society of Pesticide Science, India. 1993
8. Vijayalakshmi K et al., Neem – A User's Manual, Centre for Indian Knowledge Systems, India. 1995
9. Wandscheer CB, Duque JE, da Silva MAN, Fukuyama Y, Wohlke JL, Adelmann J, Fontana JD (2004) Larvicidal action of ethanolic extracts from fruit endocarps of Melia azedarach and Azadirachta indica against the dengue mosquito Aedes aegypti. Toxicon 8:829–835.
10. Barnett RB, Dr. Jeanine Barone J, Ayurvedic Medicine – Ancient Roots, Modern Branches, Concorp Management, USA. 1996
11. Puri HS, Neem the Divine Tree, Harwod Academic Publishers. 1999
12. Gahukar RT, Neem in Plant Protection, Agri-Horticultural Publishing House, 1995
13. Singh RP et al., Neem & Environment, Oxford & IBH Publishing Co. Pvt. Ltd. 1996
14. Kabra KN, Development & Ecological Role of Neem in India, Neem Foundation, India. 2000
15. Luo X, MaY, Wu S, Wu D Two novel azadirachtin derivatives from Azadirachta indica. J. Nat. Prod. 1999; 62: 1022-1024.
16. Mordue AJ, Blackwell A (2003) Azadirachtin: an update. J Insect 544 Physiol 39:903–924 545.

17. Prakash Srivastava RP, Neem and Pest Management, International Book distributing Co., 2001
18. Amorose T (1995) Larvicidal efficacy of neem (Azadirachta indica) oil and defatted cake on Culex quinquefasciatus Say. Geobios 22:169–173
19. Mulla MS, Su T (1999) Activity and biological effects of neem products against arthropods of medicinal and veterinary importance. J Am Mosq Control Assoc 15:133–152 543.
20. Naqvi SN, Ahmed SO, Mohamed FA (1991) Toxicity and IGR (insect regularity effect) of two neem products against Aedes aegypti (PCSIR strain). Pak J Pharm Sci 4:71–76.
21. Nicoletti M, Serafini M, Aliboni A, D'Andrea A, Mariani S (2010) Toxic effects of neem cake extracts on Aedes albopictus larvae. Parasitol Res 107:89–84.
22. Okumu FO, Knols BGJ, Fillinger U (2007) Larvicidal effects of a neem (Azadirachta indica) oil formulation on the malaria vector Anopheles gambiae. Malar J 6:63–67.
23. Rao DR, Reuben R, Venugopel MS, Nagesampagi BA, Schumetterer H (1992) Evaluation of neem, Azadirachta indica, with and without water management, for the control of culicine mosquito larvae in rice-fields. Med Vet Entomol 6:315–324.
24. Wei-Hong T, Zhan-Qian S. Research on effect of four natural ultraviolet light absorbers on photostabilization of azadirachtin-A. Agric. Sci. China, 5: 885-894.
25. Boeke SJ, Boersma MG, Alink GM, van Loon JJ, van Hnis A, Dicke, M, Rietjens IM (2004) Safety evaluation of neem (Azadirachta indica) derived pesticides. J Ethnopharmacol 94:25–41
26. Gandhi M, Lal R, Sarkaranarayaman A, Baneyel CK, Sharma PL (1988) Acute toxicity of the oil from Azadirachta indica seed (neem oil). J Ethnopharmacol 23:39–51 518.