

Neem (*Azadirachta indica* A. Juss) as a Biotechnological tool: a short review

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Abstract: Neem (*Azadirachta indica* A. Juss) is a plant native from India and very adaptable to hot climates. Commonly it has been found as an ornamental plant or used in popular medicine due to its herbal properties. The current work is a short review that aimed to discuss Neem biotechnological applications in agriculture, environment, and pharmaceutical areas. The researches were performed using online databases and the papers that discussed the prospection of Neem in the agriculture area (as a fertilizer or playing plant protection against pests and disease), pharmaceutical industry (production of metabolites with biological activity), and environmental biotechnology (eco-friendly alternatives to mitigate chemical pesticide environ damage) have chosen. Based on Neem's properties is possible to find it as a fertilizer, supplement for animal feed or playing important role in plant defenses against insects (bioinsecticide) and pathogenic microorganisms (antimicrobial activity) in the agriculture field or the pharmaceutical industry with metabolites which have antitumoral, antioxidant, and anti-bactericidal activity, besides Neem has already been reported used in combination with nanoparticles (Green nanoparticles). Overall, Neem has been mentioned as a great tool for biotechnological applications in different areas.

Palavras-chave: Fertilizer-agent; animal feed supplement; bioinsecticide; green nanoparticle

Nim (*Azadirachta indica* A. Juss) como ferramenta biotecnológica: uma breve revisão

Resumo: O Nim (*Azadirachta indica* A. Juss) é uma planta nativa da Índia e muito adaptável a climas quentes. Comumente, tem sido encontrada como planta ornamental ou usada na medicina popular devido às suas propriedades medicinais. Neste trabalho, objetivamos realizar uma breve revisão discutindo a aplicação biotecnológica do Nim nas áreas da agricultura, ambiental e farmacêutica. As pesquisas foram realizadas utilizando bancos de dados *online* e os artigos que discutiram a prospecção do Nim na agricultura (como fertilizante ou atuando na proteção de plantas contra pragas e doenças), na indústria farmacêutica (produção de metabólitos com atividade biológica) e biotecnologia ambiental (alternativas sustentáveis para amenizar os danos ambientais causados por pesticidas químicos) foram selecionados. Com base nas propriedades do Nim, é possível encontrá-lo como fertilizante, suplemento para a alimentação animal ou desempenhando papel importante nas defesas das plantas contra insetos (bioinseticida) e micro-organismos patogênicos (atividade antimicrobiana) no campo agrícola ou na indústria farmacêutica com metabólitos que possuem atividade antitumoral, antioxidante e antibacteriana, além de já ocorrer relatos do Nim em combinação com nanopartículas (“nanopartículas verdes”). No geral, o Nim tem sido descrito como uma ótima ferramenta para aplicações biotecnológicas

em diferentes áreas.

Palavras-chave: Agente fertilizante; suplemento alimentar animal; bioinseticida; nanopartículas verdes.

Como citar este artigo:

FERREIRA, A. P.; MENDONÇA, D. E. A.; OLIVEIRA, J. A. S.; CONTE, H. Neem (*Azadirachta indica* A. Juss) as a biotechnological tool: a short review. **Luminária**, União da Vitória, v.21, n.02, p. 28 – 35, 2019.

INTRODUCTION

Azadirachta indica (A. Juss) popularly known as Neem, is a plant native from India and very adaptable to hot climates and infertile sites, with extremely bitter fruits and leaves. Commonly it has been found as an ornamental plant or used in popular medicine due to its herbal properties (JINDAL et al., 1999; GAJALAKSHMI; ABBASI, 2004; KUMAR et al., 2018).

A. indica belongs to the Meliaceae family. It is a moderate to large perennial vascular plant from South and Southeast Asia. It grows from 5 to 20 m, even in regions with less than 150 mm precipitation and in regions of the temperate and tropical climate. Its propagation occurs by seed, neem seedlings aged 9-12 months can be transplanted (LOKANADHAN et al., 2012; EL-HAWARY et al., 2013).

Its trunk is straight with long branches, forming a broad round crown when extended. Its bark is rough dark brown with longitudinal cracks separated by flat furrows, and pinnate alternate-leaves with approximately 5-20 serrated leaflets, dark green when mature. These plants are capable to grow in soils with a pH ranging from 5.0 to 8.0, however, the optimum pH is around 6.2 and 7.0 in deep and permeable sandy soils (CSURHES, 2008).

The petiole is cylindrical and long with broad base and green, with flowering panicles in the armpits and leaves. They produce yellow, ellipsoid, and glabrous small drupes (12-20 mm). Its flowers are hermaphrodite, small, and white. The fruits are green and yellowish in ripening. Of the total fruits produced, about 37 to 50 kg per year, around 60% produced dry fruits with 48% of pulp, 25% of the husk, 23% of kernel husk, and

4.5% of seeds. Besides that, using the kernel it is possible to produce 55% of neem cake and 45% of Neem oil (LOKANADHAN et al., 2012).

The Neem tree, especially seeds, bark, and leaves, has been mentioned to have anti-fungal, anti-inflammatory, anthelmintic, anti-malarial, antipyretic, antioxidant, antiulcer, antiviral, diuretic, hypoglycemic, and immunostimulant properties (EL-HAWARY et al., 2013). In Asia and Africa, Neem is commonly used for the treatment of various acute and chronic disorders. Furthermore, this plant has been reported showing insecticidal activity and is widely used as a blood pressure regulator and in the alternative treatment of some gastrointestinal diseases (GUPTA et al., 2017). Regarding its environment and agricultural properties, studies have been reporting the use of *A. indica* for reforestation around the world, as in Africa, America, and Asia, because it has fast growth around 80 cm/year and can survive in dry and nutrient-poor soils (PUNTI, 2000).

According to the SCOPUS database (<https://www.scopus.com>; using “*Azadirachta indica*” as the keyword, limiting to articles as document type), between 2010 and 2020, there has been 3.105 works published so far. Among these works, approximately 27.5% of the works were developed for the Agricultural and Biological Sciences, followed by Pharmacology, Toxicology and Pharmaceutics (~ 14.1%), and Biochemistry, Genetics and Molecular Biology (11%) (Figure 1).

In this perspective, the current work is a short review that aimed to discuss Neem’ biotechnological applications in agriculture, environment, and pharmaceutical areas.

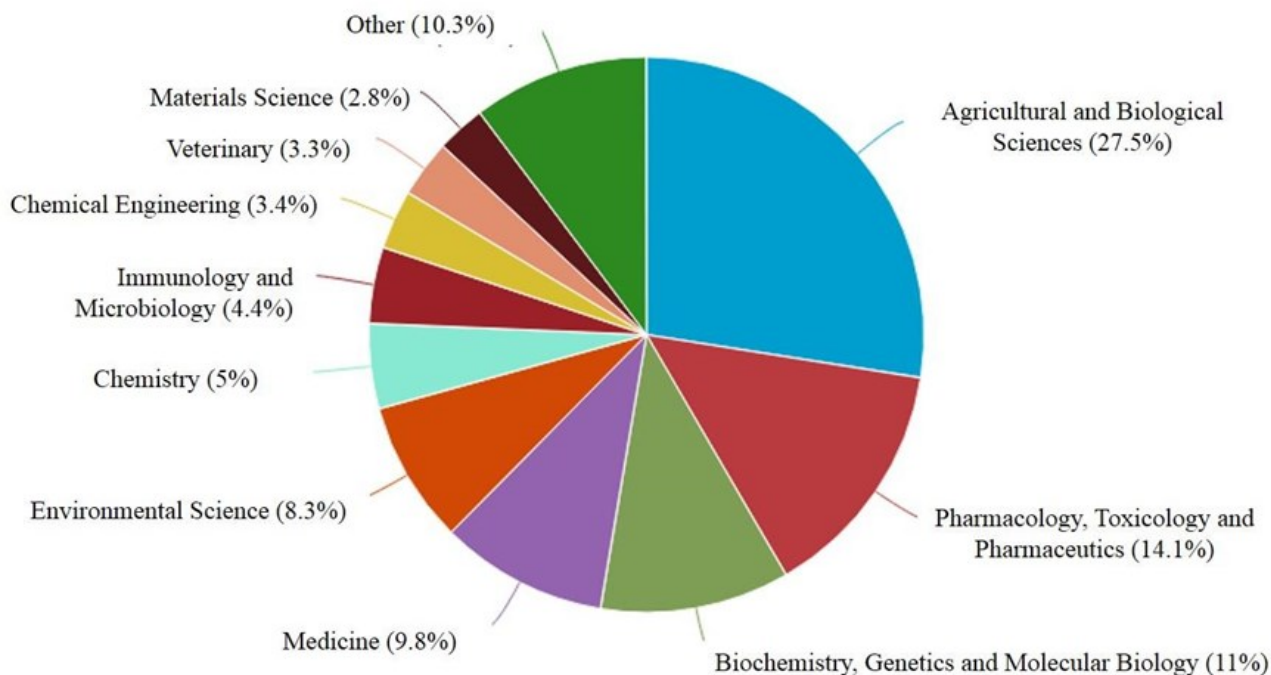


Figure 1. Research performed on SCOPUS (<https://www.scopus.com>) database 2010 to 2020 using “*Azadirachta indica*” as the keyword, limiting to articles as document type). Accessed on September 27, 2019.

MATERIAL AND METHODS

The researches were performed using online databases such as Google Scholar (<https://scholar.google.com.br/>), PubMed (<https://www.ncbi.nlm.nih.gov/pubmed/>), Science Direct (<https://www.sciencedirect.com/>), Scielo (<https://scielo.org/>), and SCOPUS (<https://www.scopus.com>). The keywords used were “Neem”, “*Azadirachta indica*”, “Neem properties” and “Neem biotechnology application”, limiting the years from 1999-2019.

Through the five research online platforms, approximately ten thousand works were found using the descriptors mentioned above in the last 20 years. Among these, approximately thirty-two works prospecting Neem in the agricultural area (as a fertilizer-agent or protecting plants against pests and diseases), in the pharmaceutical industry (production of metabolites with biological activity), in environmental biotechnology (ecological alternatives to mitigate environmental damage to chemical pesticides), and of the development of nanoparticles were chosen.

RESULTS AND DISCUSSION

Bioactive compounds from Neem

Different compounds with biological activity have been reported obtained from *A. indica* as alkaloids, flavonoids, triterpenes, phenolic compounds, carotenoids, steroids, and ketones (SANTOS; ANDRADE, 2000). Its branches and leaves are often used to produce essential oils and metabolic extracts employing different organic solvents. As an example, we can mention the triterpenoids obtained from these plants. Triterpenoids belong to terpene’s group and have been described as playing an anti-inflammatory and antimicrobial activity (GUPTA et al., 2017).

Through the Neem tree, it is possible to obtain different substances, and a very important compound among the properties of this plant are oils. The oil contains around 300 biologically active compounds, most notably azadiractin - a triterpene (PASCOLI et al., 2019). In the oil withdrawn from the root of *A. indica*, there are six different types of limonoids (17-hydroxy-sandaracopimar-8,15-dien-11-one, diterpene, nimbinene, nimbinal, nimbadiol, and salanine) and three diterpenoids (nimbidiol, ferruginol, and 6,7-

dehydroferruginol) compounds with anti-inflammatory activity, antimycobacterial action, and cytotoxic properties (PASSOS et al., 2019).

The advancement of cosmetic technology, oils extracted from Neem have been used to obtain products with social interest. An example was the development of soap from Neem seed oil by researchers Mark-Mensah and Firempong (2011). Their research has shown that Neem oil can be used as a palm oil substitute in soap production and still be a cosmetic with favorable medicinal properties.

Antitumoral activity

Many studies have been assayed anti-tumor action of Neem metabolic extracts against tumor cells. Kumar et al. (2006) have obtained an ethanolic extract from Neem's leaves. These authors studied cell growth and *in vitro* ethanolic extract action against prostate cancer cells (PC-3). There were related by Kumar et al. (2006) positive results against these cancer cells suggesting the Neem potential to find new and unexplored compounds for the pharmaceutical industry, especially for prostate cancer treatment.

Another study looking for Neem's anti-tumor properties was carried out by He et al. (2016). These researches evaluated metabolites obtained from this plant against hepatic tumor cells. After the evaluation in mice, the author's findings showed promising results, demonstrating that Neem extract has cytotoxicity against the hepatic tumor cells without causing any damage to the mice's normal cells. These studies demonstrate the potential of Neem as an antitumor-agent, however, more detailed studies are required to a better understanding of the mechanism of action of the compounds produced by it.

Bioinsecticidal activity

Pesticides, such as insecticides, and chemical defensives are frequently used to insect control in integrated pest management. However, the uncontrolled use of these chemical agents has been bringing environmental damages, making necessary the development of new agents or strategies that can be as efficient as a chemical insecticide, but

more eco-friendly and sustainable (GUAN et al., 2014).

Pesticide values used worldwide during seven years (2010-2017) are described in Table 1. It is observed there were produced around 32 million tonnes of pesticides and spent around U\$ 251 million dollars, which means the trade of these chemical substances is very expensive, besides being too dangerous for the environment.

In this perspective, there are natural alternatives to obtain insecticides by plants and microorganisms, which the discovery is

Table 1. Pesticide use in tonnes and trade-in U\$ of pesticides worldwide between 2010 to 2017 according to FAOFAST by Food and Agriculture Organization of the United Nations. Data obtained from FAOFAST. Available on <http://www.fao.org/faostat/en/>. Accessed on September 27, 2019.

Year	Pesticide use (tonnes)	Pesticide trade (U\$)
2010	3.961.986	24.535.748
2011	4.052.029	29.422.812
2012	4.093.183	30.219.981
2013	4.045.838	33.647.667
2014	4.105.783	35.666.286
2015	4.061.364	32.282.964
2016	4.088.247	31.525.722
2017	4.113.591	34.270.513
Total	32.522.021	251.571.693

more sustainable when compared to chemical pesticides, without their mutagenic and toxic effects on living beings and the environment (GUAN et al., 2014; YING, 2018).

Among the alternatives, one of them is Neem, whose action of the bioinsecticide-agent is effective in controlling mosquitoes that transmit human diseases such as *Aedes* spp., *Anopheles gambiae*, *Anopheles culicifacies*, *Bamisia tabaci*, *Culex quinquefasciatus*, and some arthropods (MULLA; SU, 1999; SU; MULLA, 1999; NDION et al., 2007; OKUMU et al., 2007; KALA et al., 2019).

So far, the Neem has proven efficiency in 550 species of insect pests and is also used as an antiparasitic, fungicide, and nematicide

(MONDAL; MONDAL, 2012; KUMARI et al., 2019). It is believed that this activity may be related to its antimetabolic action, larval cuticle damage (preventing its removal), toxicity and deleterious effects on the endocrine system, sterilization, fecundity, growth regulation, oviposition repellency, and suppression (MULLA; SU, 1999).

An example, when Neem is used as an insecticide in rice fields, it is effective in increasing crop productivity and controlling pests (KAMARULZAMAN et al., 2018). In addition, Neem products do not leave residues in the environment, and they are quickly degraded by the action of abiotic agents like sunlight, high temperatures, and pH (NICOLETTI et al., 2012).

Neem as a biotechnological tool

Based on the extraction of different bioactive oils from Neem, it is possible to apply them as fertilizers and thus can improve soil quality for better agricultural production (PASCOLI et al., 2019). In this context, a study by Gajalakshmi; Abbasi (2004) aimed to explore Neem leaf substrates to generate vermicomposts and to observe its impact on the growth and production of *Solanum melongena* (eggplant plants). It was observed that the plants treated with vermicompost obtained from Neem leaf increased significantly their height and biomass, beef up the flowering and fruit production when compared with plant control.

Among all the characteristics and applicability that *A. indica* has, another one is its use in the production of organic supplements for animal feed. Musalia et al. (2000) investigated the possibility of replacing peanuts with urea-treated Neem seed cake (as the main protein source) in a concentrated mixture present in the feeding of growing lambs. They obtained a satisfactory result, but there should be more studies to better understand the functioning of this type of supplement.

Neem could also be used in combination with nanoparticles. Nanoparticle, by definition, has a size equal to or smaller than 100 nm and may be presented through different materials such as cobalt, silver, gold, and others. The production of these "conventional"

nanoparticles is a big process, being very expensive and involving substances with a worrying toxicity level (OLIVEIRA et al., 2019). In this way, biological alternatives have been developed, giving rise to nanoparticles from plants or part of them and thus helping in a friendly process (TRIPATHY et al., 2010). As a nanoparticle, Neem has already been tested in the treatment of acne as non-pesticide, antimicrobial activity, antitumor activity and improving cotton fabrics (VIJAYAN et al., 2013; VERMA; MEHATA, 2016; MATTOS, et al., 2017; SUBRAMANI et al., 2017; PASCOLI et al., 2019; SIDDIQ et al., 2019).

CONCLUSIONS

Many plants are known for their medicinal properties, such as Neem. Neem is capable of producing several classes of compounds with biotechnological applications and industrial interest in different areas such as for the pharmaceutical sector producing substances capable of controlling the proliferation of cancer cells, activity has already been demonstrated *in vitro* in tumor cells and *in vivo* in mice. Further, some substances produced by Neem can control vectors that disseminate arboviruses like Dengue, Zika, and Chikungunya and are of great importance not only for the pharmaceutical sector but also for the socio-economic area.

Another important application of Neem is related to the production of biologically active molecules against insect pests and plant pathogenic microorganisms, favoring more sustainable agricultural practices, decreasing the application of chemical pesticides that are often toxic to the environment. Given these perspectives, Neem presents itself as a great source to be explored with positive results for the scientific area and economic/social development.

ACKNOWLEDGMENTS

We would like to thank Universidade Estadual de Maringá, CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support.

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Submetido em: 02 de setembro de 2019.

Aceito em: 01 de junho de 2020.