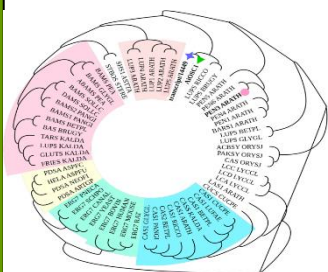


Neem Research Newsletter

Volume 2, Issue 8, 2022



WORLD NEEM ORGANISATION (WNO)



From
The Editor's Desk.....

Greetings! This issue of the newsletter showcases abstracts of several research articles on neem in diverse fields. There is increased emphasis on nanoformulations of neem with enhanced efficacy as biopesticides, antibacterial agents and to control vector-borne disease insects. Experiments in India have demonstrated that using neem-coated urea can have a major impact on transforming agriculture climate-smart. Findings from Bangladesh highlight the potential of neem as a biofuel feedstock. Biofuel production from neem seeds have been evaluated via non-catalytic and catalytic pyrolysis process. Neem cake cellulose was found to be suitable to be incorporated as a biofiller material in polymer matrices to manufacture eco-friendly composites because of its high cellulose content, better crystallinity, lower density, thermal stability, kinetic activation energy, particle size, and good surface roughness. The antiviral, antiparasitic, anti-inflammatory, antiproliferative effects have been documented besides its protective effects against liver injury and skin ulcers. Addition of neem leaf powder to the diet was found to a promising phytogetic supplement for growing lambs with no detrimental effects.

S. Nagini

Core Founding Member, WNO
Chief Scientific Coordinator &

Regional Director, South India



Neem in Agriculture

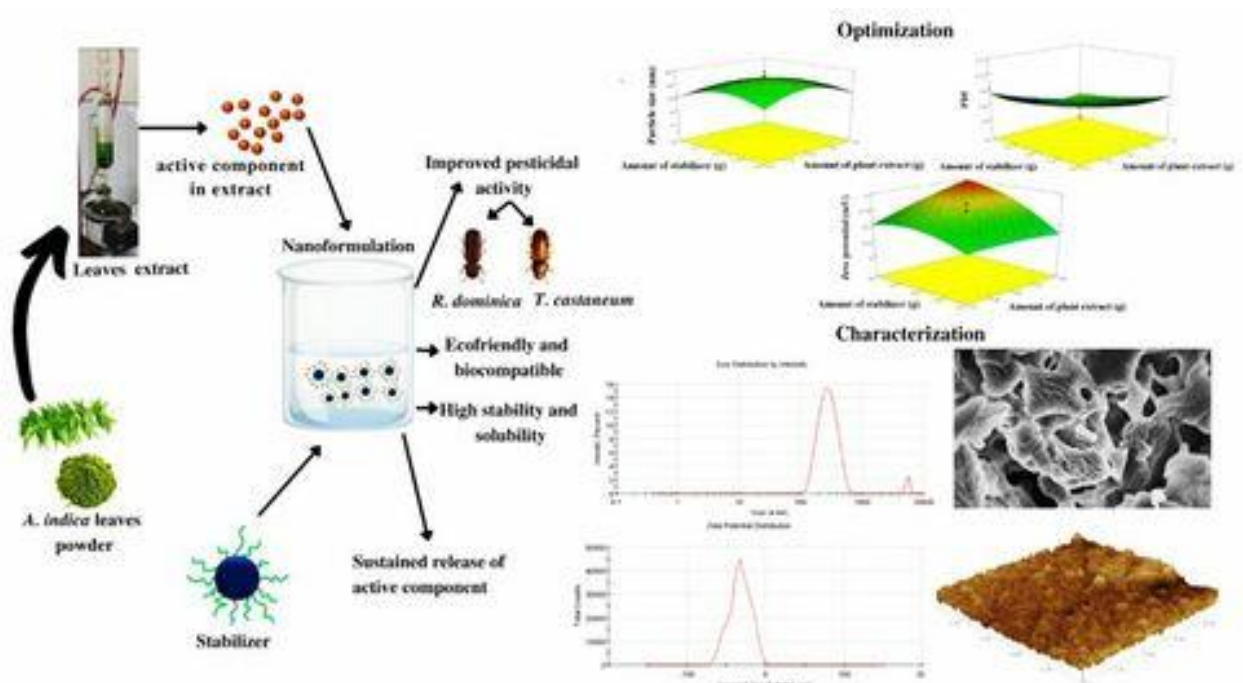
Formulation and Characterisation of *Azadirachta indica* Nanobiopesticides for Ecofriendly Control of Wheat Pest *Tribolium castaneum* and *Rhyzopertha dominica*.

Iqbal H, Jahan N, Ur-Rehman K, Jamil S.

J Microencapsul. 2022 Nov 18:1-44. doi: 10.1080/02652048.2022.2149870. Online ahead of print. PMID: 36398734

Aim: This study aimed to formulate the green, sustainable, and ecofriendly nanobiopesticides of *Azadirachta indica* with enhanced pest control efficacy.

Methods: Nanoprecipitation method was used for the development of nanobiopesticides. Optimisation was done by response surface methodology. Nanoformulations were characterised by zetasizer, scanning electron microscopy, energy dispersive x-ray spectroscopy, atomic force microscopy, and fourier transform infrared spectroscopy. Pesticidal potential of nanosuspensions was evaluated by insecticide impregnated filter paper method. **Results:** Optimised nanobiopesticide showed an average particle size of $275.8 \pm 0.95\text{nm}$, polydispersity index 0.351 ± 0.002 , and zeta potential of $-33 \pm 0.90\text{mV}$. Nanobiopesticides exhibited significantly higher mortality rates of 86.81 ± 3.04 and $84.97 \pm 2.83\%$ against *Tribolium castaneum* and *Ryzopertha dominica* respectively as compared to their crude extract. Minor change in particle size from 275.8 ± 0.95 to $298.8 \pm 1.00\text{nm}$ and polydispersity index from 0.351 ± 0.002 to 0.445 ± 0.02 were observed after three months of storage at 4°C . **Conclusions:** Pesticidal efficacy of *Azadirachta indica* was significantly enhanced by the formulation of its nanobiopesticides.



In Vitro Antibacterial Activity of Green Synthesized Silver Nanoparticles Using *Azadirachta indica* Aqueous Leaf Extract against MDR Pathogens.

Alqahtani O, Mirajkar KK, Kumar K R A, Mahnashi MH, Shaikh IA, Mitra S, Nagaraj H, More SS, Muddapur UM, Khan AA, Sudarshan PR.

Molecules. 2022 Oct 25;27(21):7244. doi: 10.3390/molecules27217244.PMID: 36364070

Rice is the most important staple food crop feeding more than 50% of the world's population. Rice blast is the most devastating fungal disease, caused by *Magnaporthe oryzae* (*M. oryzae*) which is widespread in rice growing fields causing a significant reduction in the yield. The present study was initiated to evaluate the effect of green synthesized silver nanoparticles (AgNPs) on the biochemical constituents of rice plants infected with blast. AgNPs were synthesized by using *Azadirachta indica* leaf extract and their characterization was performed using UV-visible spectroscopy, particle size analyser (PSA), scanning electron microscope (SEM), and X-ray diffraction (XRD) which confirmed the presence of crystalline, spherical shaped silver nanoparticles with an average size of 58.9 nm. After 45 days of sowing, artificial inoculation of rice blast disease was performed. After the onset of disease symptoms, the plants were treated with AgNPs with different concentrations. Application of nanoparticles elevated the activity of antioxidative enzymes such as superoxide dismutase, catalase, peroxidase, glutathione reductase, and phenylalanine ammonia-lyase compared to control plants, and total phenol and reducing sugars were also elevated. The outcome of this study showed that an increase in all biochemical constituents was recorded for *A. indica* silver nanoparticles-treated plants. The highest values were recorded in 30 ppm and 50 ppm AgNPs-treated plants, which showed the highest resistance towards the pathogen. Green synthesized AgNPs can be used in future for disease control in susceptible varieties of rice. The synthesized AgNPs using *A. indica* leaf extract have shown promising antibacterial activity when tested against 14 multidrug-resistant (MDR) bacteria comprising Gram-negative bacteria *Escherichia coli* (n = 6) and *Klebsiella pneumoniae* (n = 7) with a good zone of inhibition diameter, tested with the disc diffusion method. Based on these findings, it appears that *A. indica* AgNPs have promise as an antibacterial agent effective against MDR pathogens.

Evaluation of Insecticidal Potentials of Five Plant Extracts against the Stored Grain Pest, *Callosobruchus maculatus* (Coleoptera: Bruchidae).

Akbar R, Khan IA, Alajmi RA, Ali A, Faheem B, Usman A, Ahmed AM, El-Shazly M, Farid A, Giesy JP, Aboul-Soud MAM.

Insects. 2022 Nov 13;13(11):1047. doi: 10.3390/insects13111047.PMID: 36421950

Plant based insecticides are considered among the most economic and ecofriendly chemicals for the protection of plants and stored grains. The cowpea weevil (*Callosobruchus maculatus*) causes more than 90% damage to stored grains in three to six months. The current study investigates insecticidal potentials of five selected botanicals: *Melia azedarach*, *Nicotiana rustica*, *Azadirachta indica*, *Nicotiana tabacum* and *Thuja orientalis*. They are explored at six different concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%) against *C. maculatus* and compared to effects of distilled water which is used as a control. Toxicities of 3%(V/V) extracts of *N. tabacum*, *N. rustica*, *A. indica* and *T. orientalis* against *C. maculatus* were 100%, 86.11%, 80.56% and 72.22%, respectively. Maximum mortality was caused by *N. tabacum* and *N. rustica* (100%), followed by *A.*

indica (82%), whereas minimum mortality was observed in *T. orientalis* (64%) at 2.5%. Several phytochemicals, alkaloids, saponins, diterpenes, phytosterol, flavonoids and phenols were identified in *N. tabacum* and *N. rustica*, while few were present in *A. indica*. Phytosterol was present in greatest abundance. Saponins were only detected in aqueous extracts of *N. rustica* and *N. tabacum*. Taken together, these results indicate the utility of *N. tabacum*, *N. rustica* and *A. indica* as potential botanicals to control pest beetle and cowpea weevil.

Reduced dose coupled with improved method of neem-coated urea application and foliar sprays of KNO₃ enhance cotton yield and fiber parameters under semi-arid climates.

K Singh, SK Mishra, P Rathore, A Kumar

Journal of Plant Nutrition, 2022. <https://doi.org/10.1080/01904167.2022.2144368>

Choice of source, nitrogen rate, and its application method affect cotton productivity. In absence of new recommendations for recently introduced neem-coated urea (NCU) in India, farmers have been applying either similar or above optimal levels of NCU in cotton just like conventional urea. The present study aimed to evaluate the optimum dose and better method of NCU application to improve cotton productivity and profitability under arid climates. A total of eight different treatments were evaluated in a randomized complete block design. Two NCU doses [i.e., 112.5 kg ha⁻¹ (75% of conventional nitrogen [urea] recommendation-CNR) and 150 kg ha⁻¹ (100% of CNR)] were applied using three methods, i.e., (a) band application, (b) spot application, and (c) broadcasting constituted six treatments. In addition, foliar sprays of NCU@1% and KNO₃@2% were also evaluated to study their effect in improving seed cotton yield (SCY) and fiber parameters. These findings elucidated that the spot method of NCU application proved better than the broadcasting and band application method owing to better nitrogen use efficiency (NUE). NCU@ 112.5 kg N ha⁻¹ applied through spot application along with three foliar sprays of KNO₃@2% resulted in similar profitability as that of broadcasting 150 kg NCU ha⁻¹ along with four foliar sprays of KNO₃. Improved physiological parameters like net photosynthesis and chlorophyll coupled with higher NUE played a decisive role for at par SCY despite 25% low nutrient input besides enhanced environment safety. Therefore, under semi-arid climates, 37.5 kg N ha⁻¹ could be saved without compromising cotton productivity.

Impact, adaptation, and mitigation of climate change in Indian agriculture.

Pathak H.

Environ Monit Assess. 2022 Nov 1;195(1):52. doi: 10.1007/s10661-022-10537-3. PMID: 36316531

Climate change poses serious risks to Indian agriculture as half of the agricultural land of the country is rainfed. Climate change affects crop yield, soil processes, water availability, and pest dynamics. Several adaptation strategies such as heat- and water stress-tolerant crop varieties, stress-tolerant new crops, improved agronomic management practices, improved water use efficiency, conservation agriculture practices and improved pest management, improved weather forecasts, and other climate services are in place to minimize the climatic risks. The agriculture sector contributes 14% of the greenhouse gas (GHG) from the country. Mitigation of GHG emission from agriculture can be achieved by

changing land-use management practices and enhancing input-use efficiency. Experiments in India showed that methane emission from lowland rice fields can be reduced by 40-50% with alternate wetting and drying (AWD), growing shorter duration varieties, and using neem-coated urea according to soil health card (SHC) and leaf color chart (LCC). Dry direct-seeding of rice, which does not require continuous soil submergence, can reduce methane emission by 70-75%. Sequestration of carbon (C) in agricultural soil can be promoted with the application of organic manure, crop residues, and balanced nutrients. India has taken several proactive steps for addressing the issues of climate change in agriculture. Recently, it has also committed for reducing GHG emission intensity by 45% by 2030 and achieving net zero emission by 2070. The paper discusses the major impacts of climate change, potential adaptation, and mitigation options and the initiatives of Govt. of India in making Indian agriculture climate-smart.

Nutritional and biochemical response of neem to seed applied bioinoculants under nursery conditions

L Singh, R Sharma, R Chhabra, R Kaur, S Sharma

Range Management Soc India 2022, vol 43. No. 2.

The present investigation was conducted with an aim to decipher the effect of seed applied bioinoculants on nutritional status of neem seedlings and biological properties of growing media under nursery conditions. Healthy neem seeds were collected from trees growing in university campus and given five treatments viz., Azotobacter (nitrogen fixer; T₁), Pantoea agglomerans (plant growth promoting rhizobacteria; T₂), Pseudomonas fluorescens (phosphorus solubilizing bacteria; T₃), consortium 1 (PAU recommended; T₄), consortium 2 (T₁+T₂+T₃; T₅) and control to record their effect on survival percentage and nutrient content viz., N, P and K after 3 and 6 months. Soil attributes of the growing media along with chlorophyll and total soluble sugars from leaves were estimated after 3 and 6 months. Application of consortium 2 increased survival percentage of neem seedlings along with nutrient content of root, shoot and leaf portions of neem seedlings. Available P, K, organic carbon, alkaline phosphatase and dehydrogenase activity were also found higher in soils treated with microbial consortium 2. Among all treatments, consortium 2 reflected maximum nutrient content accumulation in all parts of seedlings. Soil analysis also revealed better rhizospheric conditions in terms of available phosphorus, potassium content and enzymatic activity. This study endorsed the positive impact of bioinoculants application on better performance of neem seedlings.

Resistance to Beta-cypermethrin, Azadirachtin, and Matrine, and Biochemical Characterization of Field Populations of Oedaleus asiaticus (Bey-Bienko) in Inner Mongolia, Northern China.

Gao S, Tan Y, Han H, Guo N, Gao H, Xu L, Lin K.

J Insect Sci. 2022 Nov 1;22(6):1. doi: 10.1093/jisesa/ieac063.PMID: 36374481

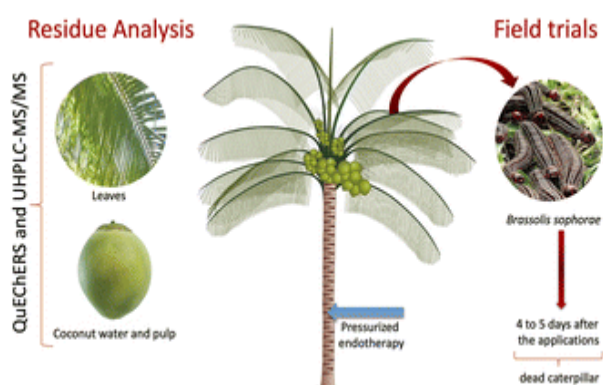
Oedaleus asiaticus (Bey-Bienko) is an economically devastating locust species found in grassland and pastoral areas of the Inner Mongolia region of northern China. In this study, resistance to three frequently used insecticides (beta-cypermethrin, matrine, and azadirachtin) was investigated in six field populations of O. asiaticus using the leaf-dip bioassay method. The inhibitory effects of synergists and the activities of detoxification

enzyme activities in the different populations were determined to explore potential biochemical resistance mechanisms. The results showed that the field populations SB (resistance ratio [RR] = 7.85), ZB (RR = 5.64), and DB (RR = 6.75) had developed low levels of resistance to beta-cypermethrin compared with a susceptible control strain. Both the SB (RR = 5.92) and XC (RR = 6.38) populations had also developed low levels of resistance against matrine, with the other populations remaining susceptible to both beta-cypermethrin and matrine. All field populations were susceptible to azadirachtin. Synergism analysis showed that triphenyl phosphate (TPP) and diethyl-maleate (DEM) increased the toxicity of beta-cypermethrin significantly in the SB population, while the synergistic effects of TPP, piperonyl butoxide (PBO), and DEM on the toxicity of matrine were higher in SB (SR 3.86, 4.18, and 3.07, respectively) than in SS (SR 2.24, 2.86, and 2.29, respectively), but no synergistic effects of TPP, PBO, and DEM on azadirachtin were found. Biochemical assays showed that the activities of carboxylesterases (CarEs) and glutathione-S-transferases (GSTs) were significantly raised in all field populations of *O. asiaticus*, with a significant positive correlation observed between beta-cypermethrin resistance and CarE activity. The activities of cytochrome P450 monooxygenases (P450) and multi-function oxidases (MFO) were elevated in all six field populations, and P450 activity displayed strong positive correlations with the three insecticides. Our findings suggest that resistance to beta-cypermethrin in *O. asiaticus* may be mainly attributed to elevated CarE and GST activities, while P450 plays an important role in metabolizing matrine and azadirachtin. Our study provides insights that will help improve insecticide resistance management strategies.

Study of insecticide translocation in coconut palm trees after using pressurized endotherapy.

Ferreira JA, Almeida GB, Lins PMP, Tavares MM, Farias SCC, Queiroz SCN. *Anal Methods*. 2022 Nov 18. doi: 10.1039/d2ay01328b. PMID: 36398780

Alternative techniques for applying agricultural products, such as pressurized endotherapy, have shown promise in pest and disease control in coconut palms (*Cocos nucifera* Linn.). In this work, azadirachtin and abamectin were applied by pressurized endotherapy to control *Brassolis sophorae*. Twelve different treatments were carried out, and in all of them, at least one plant had the larvae completely dead four and five days after endotherapeutic applications, and all the others died within the next few hours. Leaf and fruit analyses were performed to determine the concentration over time. High concentrations of abamectin were observed after 15 and 30 days on leaves when applied in larger volumes. In fruits, no residue was found regardless of the applied concentration. Analytical methods were developed and validated for leaves and fruits to analyze insecticide residues using LC-MS/MS and modified QuEChERS acetate according to SANTE/11813/2017 guidelines. The insecticide translocation tests in the leaves and the high mortality of insects showed that pressurized endotherapy is a technique to be considered for future studies in controlling *B. sophorae* in coconut palm trees.



Development of a 'green' nanoformulation of neem oil-based nanoemulsion for controlling mosquitoes in the sustainable ecosystem

ATH Mossa, RI Mohamed, SMM Mohafrash

Biocatalysis and Agricultural Biotechnology, Volume 46, November 2022, 102541

Controlling vector borne disease insects control such as mosquitoes and reducing the use of synthetic insecticides is a major public health challenge. Green nanoinsecticides are suitable alternatives to synthetic insecticides, enabling effective and safer insect control. This study aimed to develop neem oil-based nanoemulsions using Tween 80 and/or gum arabic (GA) as a natural emulsifiers and to study their larvicidal, and adulticidal activity against *Culex pipiens*. The fatty acids in the oil were identified using gas chromatographs-mass spectrometry (GC-MS). We also studied the acute oral toxicity on male rats and its ecotoxicological effects using the Microtox® assay. We fabricated neem oil nanoemulsions with and without GA at different ratios and performed physicochemical, stability, and characterization studies. The results showed that the major fatty acids in neem oil were oleic acid (44.79%), linoleic acid (18.41%), palmitic acid (18%), and stearic acid (16.64%). We created two highly stable nanoemulsions, i.e., samples 2C and 6C with a droplet sizes of 87.4 and 146.7 nm by mixing neem oil, GA, and Tween 80 at a ratio of 1:0:1.5, and 1:1.5:0, respectively, followed by sonication for 10 min. The lethal concentration (LC₅₀) of neem oil, 2C and 6C were 9.36, 6.45, and 8.01 µg/ml for the *Culex pipiens* larvae and 21.7, 8.8 and 17.2 µg/ml for the adults. The toxicity time (T₅₀) was 40.7, 10.0, and 28.9 min for normal neem oil, samples 2C and 6C, respectively. The nanoemulsions altered the toxifying-detoxifying enzymes acetylcholinesterase (AChE) and glutathione S-transferase (GST) in *Culex pipiens* larvae without showing any toxicity in the male rats or the Microtox assay. The neem oil nanoformulations with Tween 80 or GA were highly stable and effective against larval and adult *Culex pipiens*. GA is a natural-surfactant and hence, is excellent emulsifier alternative to synthetic surfactants. The nanoemulsions did not display any toxic and ecotoxic effects. Therefore, these nanoemulsions can be considered potential alternatives to synthetic insecticides for controlling vector-borne disease insects.

Neem in Aquaculture

A blend of Guava, Bitter, and Neem Leaf extracts improves haematology and resistance to co-infection of *Streptococcus agalactiae* and *Aeromonas jandaie* but not Liver health in Nile tilapia.

Abarike ED, Dandi SO, Ampofo-Yeboah A.

Fish Shellfish Immunol Rep. 2022 Aug 18;3:100066. doi: 10.1016/j.fsirep.2022.100066.

eCollection 2022 Dec. PMID: 36419611

Given the intense interest in the use of herbal extracts to improve fish growth, fish health, and disease resistance in fish in culture systems, in this study, we examined the effects of a blend of Guava, Bitter and Neem leaf extracts (GBNL) (i.e., 1:1:1 for GL, BL, and NL respectively) at different inclusion (i.e. 0 GBNL gkg⁻¹, 1 GBNL gkg⁻¹, 3 GBNL gkg⁻¹, 5 GBNL gkg⁻¹, 7 GBNL gkg⁻¹ and 10 GBNL gkg⁻¹) levels on growth, haematology, immunity, liver toxicity and resistance to bacterial co-infections in Nile tilapia. After 8 weeks of feeding, Nile tilapia fed 3 GBNL gkg⁻¹ diets showed significant effects in improving weight gain compared to those fed the control diet. GBNL fed fish showed improved health of fish by stimulating significant increases in levels of White blood cells, Red blood cells, Haemoglobin, and Haematocrit in relation to those fed the control diet. Also, the applications of deferent GBNL levels in Nile tilapia diets showed the potential to upregulate the expression of the immune-related genes heat shock protein 70, chicken type lysozymes, and Beta-defensin, with significant effects shown in fish fed 5GBNL gkg⁻¹ diets in comparison to the control. The results also indicate that GBNL supplementation can decrease mortalities to co-infection of *Streptococcus agalactiae* and *Aeromonas jandaie* in Nile tilapia with the lowest mortalities of 13.65% and relative per cent survival of 82.57 % in fish fed 5GBNL gkg⁻¹. Despite the potential of GBNL applications in Nile tilapia, findings of this study indicate fish fed the different concentrations of GBNL, particularly with 7 GBNL gkg⁻¹ can promote the leaching of the liver enzymes: alanine transaminase, aspartate aminotransferase, and alkaline phosphate into the bloodstream which is suggestive of potential liver damage in Nile tilapia. Histological examinations of a cross-section of the liver tissues of fish fed GBNL showed various injuries including hydropic changes, pyknosis nuclei, erythrocytes congestion and vacuolation with the severest seen in those fed 7 GBNL gkg⁻¹. Taking all of the above into consideration, 5GBNL gkg⁻¹ application could improve the health and disease resistance of Nile tilapia; however, prolong use thus after 8 weeks of administration could be injurious to fish liver health.

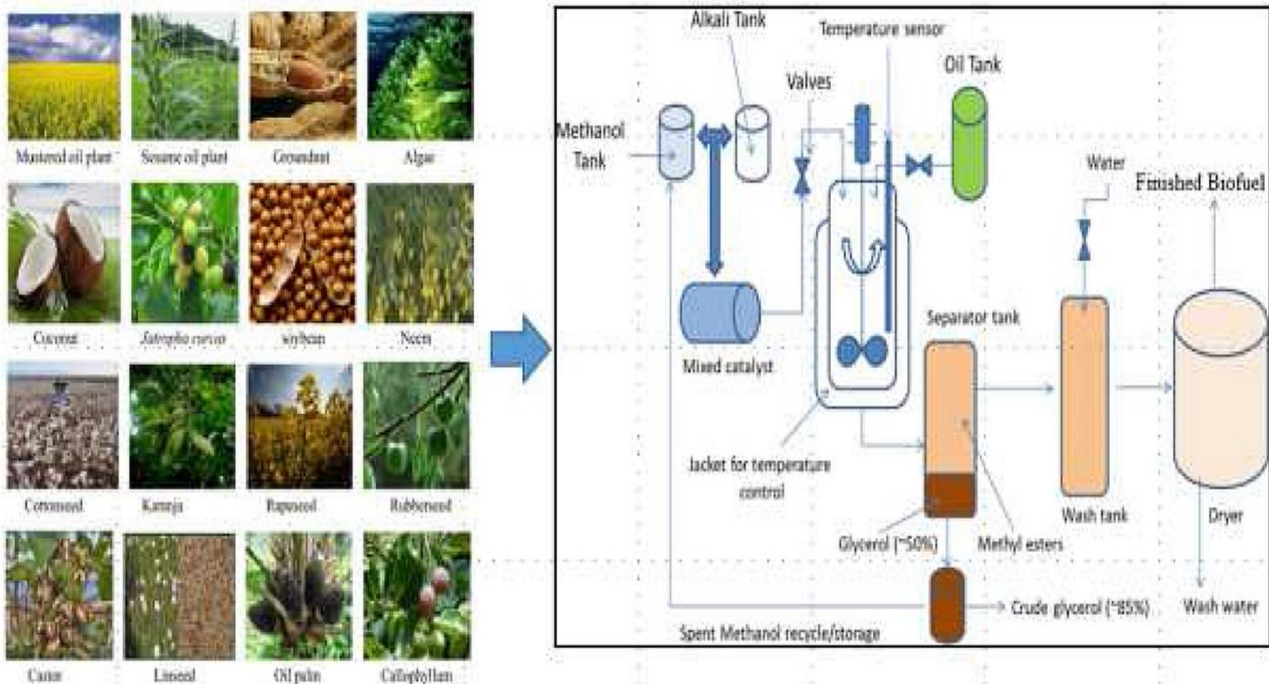
Neem- for Energy & Sustainable Environment

Potentiality of biodiesel and bioethanol production from feedstock in Bangladesh: A review.

Roy DK, Abedin MZ.

Heliyon. 2022 Oct 27;8(11):e11213. doi: 10.1016/j.heliyon.2022.e11213. eCollection 2022 Nov. PMID: 36353152

The aim of this paper is to provide a review of the most promising opportunities for sustainable biofuel generations in Bangladesh. Many researchers provide their opinions with their experimental results, but there has been no overall statistics and potentiality for the sustainable production of biofuel such as bioethanol and biodiesel in Bangladesh. The paper reviews the recent statistical conditions and the potential of biodiesel and bioethanol production in Bangladesh compared to other countries. Basically, the paper focuses on the potentiality of various biofuel feedstocks like as soybean oil, mustard oil, cottonseed oil, sesame oil, coconut oil, algae, rubber seed oil, jatropha, karanja oil, castor, bahera, neem, rice bran oil, pitraj and also different types of residues, crops, fruits, wastes. Among these reviewed papers, it is revealed that Bangladesh can generate annually about 0.16 million tons of edible oil. In addition, Bangladesh has the ability to produce about 1001881 tons of biodiesel from 2387500 tons of non-edible oil. Also, 0.04 million metric tons of biodiesel can be made from rubber seed oil. On the other hand, about 32 metric tons of bioethanol from 65.36 metric tons of agricultural crop residues, and about 143670082.36 gallons of bioethanol from 10.22 million metric tons of potato that is enough to meet the demand of 5% bioethanol blend annually. Furthermore, Bangladesh can produce 31.65 million metric tons of bioethanol from rice residue and 1.34 million metric tons of crude rice bran oil from rice husk of the paddy. It is conjectured that these annual production of various feedstocks can be used as major sources of biofuel and also can meet the demands of biofuel in Bangladesh.



Valorization of neem seeds biomass to biofuel via non-catalytic and catalytic pyrolysis process: Investigation of catalytic activity of Co-Mo/Al₂O₃ and Ni-Mo/Al₂O₃ for biofuel production.

Saidi M, Zhandnezhad A.

J Environ Manage. 2022 Nov 17;326(Pt B):116761. doi: 10.1016/j.jenvman.2022.116761. PMID: 36403462

Biofuel production from neem seeds have been evaluated via non-catalytic and catalytic pyrolysis process. Co-Mo/Al₂O₃ and Ni-Mo/Al₂O₃ industrial catalysts have been applied in upgrading process of pyrolysis oil to biofuel. The catalytic activity test revealed that these catalysts succeeded in converting fatty acids content of pyrolysis oil into low oxygen content compounds such as alcohols, alkanes, cyclic compounds, and esters via deoxygenation route. Enhancement of temperature and catalyst loading lead to increase of bio-gas production yield, significantly. The highest yield of pyrolysis oil (60.2%) was obtained at 450 °C, heating rate of 40 °C.min⁻¹ via non-catalytic pyrolysis. Using 40% catalyst loading of Ni-Mo/Al₂O₃, the content of alcohol, cyclic and alkane compounds in the bio-oil were reached 12.65%, 21.74% and 15%, respectively. The highest selectivity using 40% catalyst loading of Co-Mo/Al₂O₃ catalyst at 450 °C was related to fatty acids (62.5%), esters (18.2%) and alkanes (6.25). It is inferred that the addition of Ni to Mo causes more progress of decarbonylation and decarboxylation reactions, and the addition of Co to Mo generates more ester compounds. Sensitivity analysis indicated that the effect of Ni-Mo/Al₂O₃ catalyst through catalytic pyrolysis process was more severe than Co-Mo/Al₂O₃ catalyst.

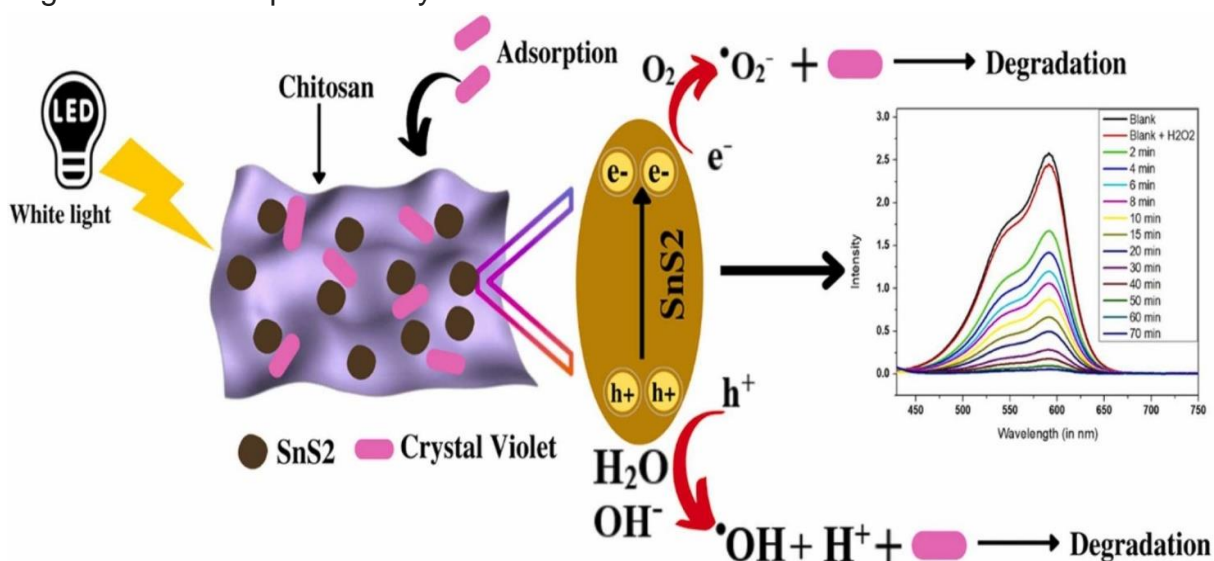
Green and environmentally sustainable fabrication of SnS₂ quantum dots/chitosan nanocomposite for enhanced photocatalytic performance: Effect of process variables, and water matrices.

Gadore V, Mishra SR, Ahmaruzzaman M.

J Hazard Mater. 2022 Nov 2;444(Pt A):130301. doi: 10.1016/j.jhazmat.2022.130301. PMID: 36403450

Concerns over the availability of clean water and the quality of treated wastewater are significant problems that call for an appropriate solution to improve the water quality. The present work emphasized the synthesis of novel SnS₂ quantum dots (QDs) deposited on chitosan via a facile green precipitation method involving neem (*Azadirachta indica*) leaf extract and investigating its photocatalytic performance for the degradation of Crystal violet (CV) dye under varying reaction parameters, other organic and inorganic salts and water matrices. The crystal structure, surface morphology, and elemental composition of the prepared SnS₂ (QDs)/Ch composite were evaluated by powder X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), photoluminescence (PL) and energy dispersive X-ray analysis (EDAX) techniques. The average size of SnS₂/Chitosan nanoparticles was calculated to be 8.8 nm using XRD, with the average diameter of SnS₂ QDs to be 3.3 nm from TEM. UV-visible spectroscopy was used to investigate its optical properties. The direct band gap of SnS₂/Chitosan estimated from Tauc's plot came to be 2.5 eV. The prepared novel SnS₂/Ch composite showed outstanding photocatalytic activity for the degradation of CV through the Advanced Oxidation Process (AOP). The fabricated photocatalyst caused

98.60 ± 1.34 % degradation of CV within a short period of 70 min under optimum conditions. The photodegradation reaction followed pseudo-first-order rate kinetics with a rate constant of 0.0815 min⁻¹. Furthermore, the photocatalyst showed high stability and was reusable for up to four cycles. The present work fulfils the aim of designing a novel, green, and efficient visible light-active nano-photocatalyst.



Chemical Modification of Neem (*Azadirachta indica*) Biomass as Bioadsorbent for Removal of Pb²⁺ Ion from Aqueous Waste Water

NA Hatiya, AS Reshad, ZW Negie

Adsorption Science & Technology, vol. 2022, Article ID 7813513, 18 pages, 2022.
<https://doi.org/10.1155/2022/7813513>

In this study, neem biomass (a mixture of neem leaf and bark), obtained from the matured neem tree, which is an eco-friendly and low-cost material was selected as a bioadsorbent to remove lead metal ion (Pb²⁺) from aqueous solutions. Neem biomass-based bioadsorbent having a carboxylic group was prepared by activation using chemical modification by NaOH and citric acid with a very simple method. The optimal activation conditions were determined as 37 min, 120°C, in 0.73 M citric acid, with a sample/acid ratio of 1/100 (mass/volume). To determine the basic properties such as chemical structure, porosity, and surface properties of the neem biomass (NB) and chemically modified neem biomass (CMNB), they were characterized by BET, FTIR, SEM, XRD, and pH_{pzc} methods. It was observed that activation has improved the adsorption capacity of the NB and also caused a more amorphous structure. The effects of adsorption parameters such as pH (2–7), contact time (10–110 min), initial Pb²⁺ ion concentration (100–300 g/L), and bioadsorbent dosage (0.1–1.1 g/L) on percentage removal of Pb²⁺ ion were studied. Maximum removal of Pb²⁺ ion (97.29%) was recorded at 0.9 g/L bioadsorbent dosage, 50 min contact time, pH of 6, and initial metal ion concentration of 100 mg/L. Kinetics and isotherm studies showed that the adsorption mechanism of Pb²⁺ ion using CMNB follows pseudosecond-order while isotherm studies fit with both models but, relatively, Freundlich model better fit having a little higher. The outcome specifies that the modified bioadsorbent can be utilized as a good and low-cost alternative for the treatment of effluent containing lead (II) ions in water.

Isolation and characterization of novel micro cellulose from *Azadirachta indica* A. Juss agro-industrial residual waste oil cake for futuristic applications

J Rantheesh, S Indran, S Raja, S Siengchin

Biomass Conv. Bioref. (2022). <https://doi.org/10.1007/s13399-022-03467-0>

Every year, the food industry generates a large amount of waste, which prompts researchers to come up with a solution to efficiently manage the issue to support zero-waste concepts. After oil extraction, many oilseed cakes remain in the oil-processing industry as a waste. Converting this oilseed cake into value-added products would reduce environmental pollution and production costs. Oilseed cakes are high in fiber and contain a lot of non-starch polysaccharides. *Azadirachta indica* A. Juss neem oil cake (NOC) is a low-cost agricultural waste material produced during the oil extraction process of neem seeds. It is a dark brown powder that contains cellulose as well as other components such as hemicelluloses, proteins, and lipids. In this investigation, cellulose was extracted from the NOC, and comprehensive characterization was carried out. The polymer composite industry is in search of biofillers to incorporate with various matrices. As neem cake cellulose (NCC) is an entirely biodegradable material, it was considered for this study. To ensure its suitability in polymer composite industries, physicochemical, morphological, thermal, and spectroscopy analyses were carried out on NCC. Higher cellulose content (73.53%), better crystallinity (66.23%), lower density (1.59 g/cm³), considerable thermal stability (335.71 °C), kinetic activation energy (83.06 kJ/mol), particle size (17.93 μm), and good surface roughness (47.004 nm) make NCC suitable to be incorporated as a biofiller material in polymer matrices to manufacture eco-friendly composites.

Neem For Human Health

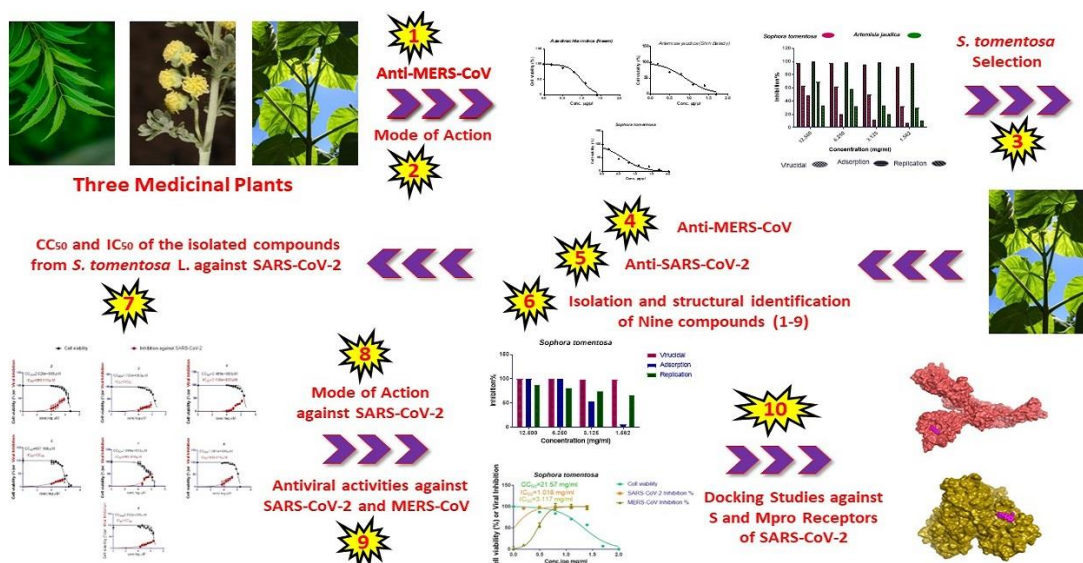
Antiviral Effects

Investigating the Potential Anti-SARS-CoV-2 and Anti-MERS-CoV Activities of Yellow Necklacepod among Three Selected Medicinal Plants: Extraction, Isolation, Identification, In Vitro, Modes of Action, and Molecular Docking Studies.

Abd-Alla HI, Kutkat O, Sweelam HM, Eldehna WM, Mostafa MA, Ibrahim MT, Moatasim Y, GabAllah M, Al-Karmalawy AA.

Metabolites. 2022 Nov 13;12(11):1109. doi: 10.3390/metabo12111109. PMID: 36422249

The anti-MERS-CoV activities of three medicinal plants (*Azadirachta indica*, *Artemisia judaica*, and *Sophora tomentosa*) were evaluated. The highest viral inhibition percentage (96%) was recorded for *S. nbsp;tomentosa*. Moreover, the mode of action for both *S. tomentosa* and *A. judaica* showed 99.5% and 92% inhibition, respectively, with virucidal as the main mode of action. Furthermore, the anti-MERS-CoV and anti-SARS-CoV-2 activities of *S. tomentosa* were measured. Notably, the anti-SARS-CoV-2 activity of *S. tomentosa* was very high (100%) and anti-MERS-CoV inhibition was slightly lower (96%). Therefore, the phytochemical investigation of the very promising *S. nbsp;tomentosa* L. led to the isolation and structural identification of nine compounds (1-9). Then, both the CC₅₀ and IC₅₀ values for the isolated compounds against SARS-CoV-2 were measured. Compound 4 (genistein 4'-methyl ether) achieved superior anti-SARS-CoV-2 activity with an IC₅₀ value of 2.13 μm. Interestingly, the mode of action of *S. tomentosa* against SARS-CoV-2 showed that both virucidal and adsorption mechanisms were very effective. Additionally, the IC₅₀ values of *S. tomentosa* against SARS-CoV-2 and MERS-CoV were found to be 1.01 and 3.11 μg/mL, respectively. In addition, all the isolated compounds were subjected to two separate molecular docking studies against the spike (S) and main protease (M^{pro}) receptors of SARS-CoV-2.



Anti-Parasitic Effects

Krimighna (anthelmintic) role of Neem Oil (medicated oil of *Azadirachta indica* Linn.) and adjuvant Ayurvedic therapies in the management of anal myiasis: A case report.
Joshi FP.

J Ayurveda Integr Med. 2022 Nov 15;13(4):100661. doi: 10.1016/j.jaim.2022.100661. PMID: 36399960

Myiasis is an uncommon pathology of parasitic infestation in live mammals by fly larvae (maggots). The fly grows and gets nourished by invading host tissue. Epidemiology of Myiasis has been recorded in tropical regions of world. No any documentation has been found on Ayurvedic intervention for anal myiasis management. The study is about a 49-year male having anal myiasis along and interno-external piles together. The case was managed by local application of Neem oil (Medicated oil of *Azadirachta indica* Linn.) soaked gauze piece once a day on daily bases in the invaded tissues of myiasis along with Ksharasutra ligation in the base of pile mass once during the treatment protocol. Internal medicines like Pippali (*Piper longum* Linn.), Erand Bhrisht Haritaki (*Terminalia chebula* Retz. roasted with castor oil) and Vidanga (*Embelica ribes* Burm. f.) were prescribed along with the local management during the course of the treatment. The case was completely cured and was relieved in 29 days. Neem oil revealed Krimighna (anthelmintic) effect which helped in tissue debridement and enhanced wound healing. Neem oil remains less irritant on local application and also gives soothing effect and kills the maggots as well.

Antibacterial & Antiproliferative Effects

Antibacterial and Antiproliferative Activities of *Azadirachta indica* Leaf Extract and Its Effect on Oil-in-Water Food Emulsion Stability.

Ouerfelli M, Metón I, Codina-Torrella I, Almajano MP.

Molecules. 2022 Nov 11;27(22):7772. doi: 10.3390/molecules27227772. PMID: 36431873

The present study aims to identify and quantify the phenolic compounds of *Azadirachta indica* leaf extract using HPLC-MS and to evaluate the antioxidant, antibacterial (against different Gram-positive and negative bacteria) and in vitro anti-proliferative activities of this extract (against breast, human liver and cervix adenocarcinoma-derived cells). The application of this extract as a natural antioxidant for food preservation was also tested on oil-in-water food emulsions for the first time in the present work in order to determine the use of *Azadirachta indica* leaves as a natural additive to preserve the food against lipid oxidation and rancidity. The results obtained revealed that 50%-aqueous ethanol leaf extract showed the best extraction yield (25.14%), which was characterized by a high content in phenolic compounds and strong antioxidant activity. Moreover, this leaf extract inhibited the growth of the bacterial strains tested (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyphi* and *Micrococcus luteus*) and showed better anti-proliferative activity against breast and cervix adenocarcinoma-derived cells than human liver cancer cells after 48 h of

treatment. Additionally, *Azadirachta indica* leaf extract showed almost similar effects as gallic acid solutions (0.25% and 0.5%) in preserving the oxidation of oil-in-water food emulsions and prevented the formation of secondary oxidation products (malondialdehyde) as well. The results obtained suggested that extracts of *Azadirachta indica* leaves are a potential source of antioxidant and antibacterial compounds and pointed to the potential of these natural extracts as therapeutic agents.

Dermatological Effects

Conservative treatment of non-healing plantar skin ulcer complicated by infection and fistula in congenital clubfoot. A case report.

Palombi L, Morelli M, Palombi M.

Ann Ital Chir. 2022 Nov 24;11:S2239253X2203866X.PMID: 36448360

Introduction: Congenital changes related to Spina Bifida (SB) include congenital clubfoot (PTC), also known as equinovarus congenital clubfoot. Skin pressure ulcers represent a frequent complication associated with SB and PTC, determined by both sensitivity deficits and skeletal alterations of plantar support. This conditions can lead to the onset of frequent neurotrophic skin ulcers. **Case report:** 72-year-old female patient suffering from spina bifida with congenital clubfoot condition, complicated by ulcerative lesion in the plantar region with fistula and infection (*Proteus Mirabilis*). An infectious disease evaluation with monitoring of the inflammatory-infectious hematochemical values and targeted antibiotic-therapy was performed. The patient performed a scintigraphic examination in order to exclude the osteomyelitis process. The dressing protocol set up was: Disinfection with disinfectant based on Poliesanide and Betaine, with the use of Nelaton 6 Fr catheter (and subsequent dressings with 18 G needle cannula), inside the fistulous channel. Subsequent abundant washing with 0.9% saline solution. Application of oily phyto-product Mix of Neem Oil and Hypericum Perforatum (1-Primary Wound Dressing), inoculating it with the catheter inside the medium and checking its leakage from both sides and cover with sterile gauze and bandage with cohesive bandage. After 4 weeks there was a reduction in the size of the fistula and the disappearance of serum-corpuseular secretions. At 7 weeks, complete re-epithelialization of the skin ulcerative lesion was observed. **Discussion:** This case report refers to the conservative medical treatment of a complex case of non-healing pressure skin ulcer with distant fistulization. The main difficulty in managing this lesion was identifying the right dressing that could reach and spread within the fistulous channel, favoring the reduction of the inflammatory-infectious process. The dressing used, as it was in an oily formulation, therefore had the right characteristics as it was easy to inoculate. The oily mix of Neem and Hypericum Perforatum (1-Primary Wound Dressing) has in fact performed a prolonged antiseptic function while maintaining the right degree of local hydration, essential for the correct carrying out of the reparative processes. **Conclusions:** In undermined or fistulous ulcers, the use of oil-based dressings, such as the oily mix of Neem and Hypericum Perforatum (1-Primary Wound Dressing), can represent a valid local therapeutic choice.

Anti-Inflammatory Effects

Nimbolide attenuates complete Freund's adjuvant induced arthritis through expression regulation of toll-like receptors signaling pathway.

Israr M, Naseem N, Akhtar T, Aftab U, Zafar MS, Faheem MA, Shahzad M.

Phytother Res. 2022 Nov 27. doi: 10.1002/ptr.7672. PMID: 36437579

Nimbolide is an active constituent of *Azadirachta indica* and is known for its anti-inflammatory, anti-oxidant, immune-modulatory, and anti-cancer effects. Few studies suggest that nimbolide treatment influences the responses to rheumatoid arthritis, but the underlying molecular mechanisms involved are not yet well established. Therefore, the present study was designed to determine the effect of nimbolide on expression regulation of toll-like receptors to attenuate rheumatoid arthritis. The rheumatoid arthritis model was established by injecting complete Freund's adjuvant (CFA) intra-dermally into the sub-plantar region of the left hind paw of rats. Nimbolide (20 mg/kg) and piroxicam (10 mg/kg) were given to arthritic rats. Rats treated with nimbolide showed a significant reduction in inflammatory cells, rheumatoid factor, ESR, and improved the body weight. The results indicated that nimbolide possesses the capacity to attenuate rheumatoid arthritis by downregulating toll-like receptors, IL-17, IL-23, HSP70, and IFN- γ expression levels. Nimbolide treatment showed significant reduction in the severity of inflammation and destruction of joints and showed comparable effects to piroxicam, which is a standard non-steroidal anti-inflammatory drug used for the treatment of rheumatoid arthritis. It can be concluded that nimbolide can be considered as a potential candidate for therapeutic targeting of the toll-like receptors pathway in rheumatoid arthritis.

Hepatoprotective Effects

A Gedunin-Type Limonoid, 7-Deacetoxy-7-Oxogedunin, from Andiroba (*Carapa guianensis* Aublet) Reduced Intracellular Triglyceride Content and Enhanced Autophagy in HepG2 Cells.

Nagatomo A, Ninomiya K, Marumoto S, Sakai C, Watanabe S, Ishikawa W, Manse Y, Kikuchi T, Yamada T, Tanaka R, Muraoka O, Morikawa T.

Int J Mol Sci. 2022 Oct 28;23(21):13141. doi: 10.3390/ijms232113141. PMID: 36361930

The seed oil of *Carapa guianensis* Aublet (Andiroba) has been used in folk medicine for its insect-repelling, anti-inflammatory, and anti-malarial activities. This study aimed to examine the triglyceride (TG) reducing effects of *C. guianensis*-derived limonoids or other commercially available limonoids in human hepatoblastoma HepG2 cells and evaluate the expression of lipid metabolism or autophagy-related proteins by treatment with 7-deacetoxy-7-oxogedunin (DAOG; **1**), a principal limonoid of *C. guianensis*. The gedunin-type limonoids, such as DAOG (% of control at 20 μ M: 70.9 \pm 0.9%), gedunin (**2**, 74.0 \pm 1.1%), epoxyazadiradione (**4**, 73.4 \pm 2.0%), 17 β -hydroxyazadiradione (**5**, 79.9 \pm 0.6%), 7-deacetoxy-7 α -hydroxygedunin (**6**, 61.0 \pm 1.2%), andirolide H (**7**, 87.4 \pm 2.2%), and 6 α -hydroxygedunin (**8**, 84.5 \pm 1.1%), were observed to reduce the TG content at lower

concentrations than berberine chloride (BBR, a positive control, $84.1 \pm 0.3\%$ at $30 \mu\text{M}$) in HepG2 cells pretreated with high glucose and oleic acid. Andirobin-, obacunol-, nimbin-, and salannin-type limonoids showed no effect on the intracellular TG content in HepG2 cells. The TG-reducing effect of DAOG was attenuated by the concomitant use of compound C (dorsomorphin), an AMPK inhibitor. Further investigation on the detailed mechanism of action of DAOG at non-cytotoxic concentrations revealed that the expressions of autophagy-related proteins, LC3 and p62, were upregulated by treatment with DAOG. These findings suggested that gedunin-type limonoids from *Andiroba* could ameliorate fatty liver, and that the action of DAOG in particular is mediated by autophagy.

Antidiabetic Effects

Known data on the therapeutic use of *Azadiracta indica* (neem) for type 2 diabetes mellitus.

Vidhya Rekha U, Anita M, Bhuminathan S, Sadhana K.

Bioinformation. 2022 Feb 28;18(2):82-87. doi: 10.6026/97320630018082. eCollection 2022. PMID: 36420434

There has been growing interest for the therapeutic use of traditional herbs in the management of diabetes mellitus (DM) and its complications. Data shows the hypoglycemic activity of *Azadiracta indica* in diabetes. Therefore, it is of interest to document known data on the therapeutic use of *Azadiracta indica* (neem) for type 2 diabetes mellitus (T2DM).

Neem for Animal Health

Effects of Neem (*Azadirachta indica*) Leaf Powder Supplementation on Rumen Fermentation, Feed Intake, Apparent Digestibility and Performance in Omani Sheep.

El-Zaiat HM, Elshafie EI, Al-Marzooqi W, Dughaisi KA.

Animals (Basel). 2022 Nov 14;12(22):3146. doi: 10.3390/ani12223146.PMID: 36428374

The objective of the present study was to evaluate the potential of the dietary addition of neem (*Azadirachta indica*) leaf powder (NLP) when compared to monensin (MON) on ruminal fermentation, feed intake, digestibility, and performance of growing lambs. Eighteen Omani lambs (22.8 ± 2.18 kg of body weight (BW)) were equally divided into three groups ($n = 6$ lambs/group) for 90 days. Animals were fed an *ad lib* basal diet consisting of Rhodes grass (*Chloris gayana*) hay (600 g/kg) and a concentrated mixture (400 g/kg) offered twice daily. Experimental treatments were control (basal diet without supplements); MON (control plus 35 mg/kg DM as a positive control); and NLP (control plus 40 g/kg DM). Lambs fed NLP had reduced ruminal ammonia nitrogen concentrations, protozoal counts, total volatile fatty acid, and blood urea nitrogen concentrations compared to the control. Compared to MON, lambs fed NLP had increased ruminal acetate and decreased propionate proportions. Inclusion of NLP in the diet increased blood total protein, globulin, and liver enzyme concentrations in comparison with the control, which was similar to MON. The lamb's final BW and average BW gain were also increased with the NLP relative to the control. Further, adding NLP to the diet increased the digestibility of crude protein compared to the control diet. In conclusion, adding NLP to the diet with 40 g/kg DM could be used as a promising phyto-genic supplement for growing lambs with no detrimental effects on the ruminal fermentation profile, nutrient intake, or digestibility.

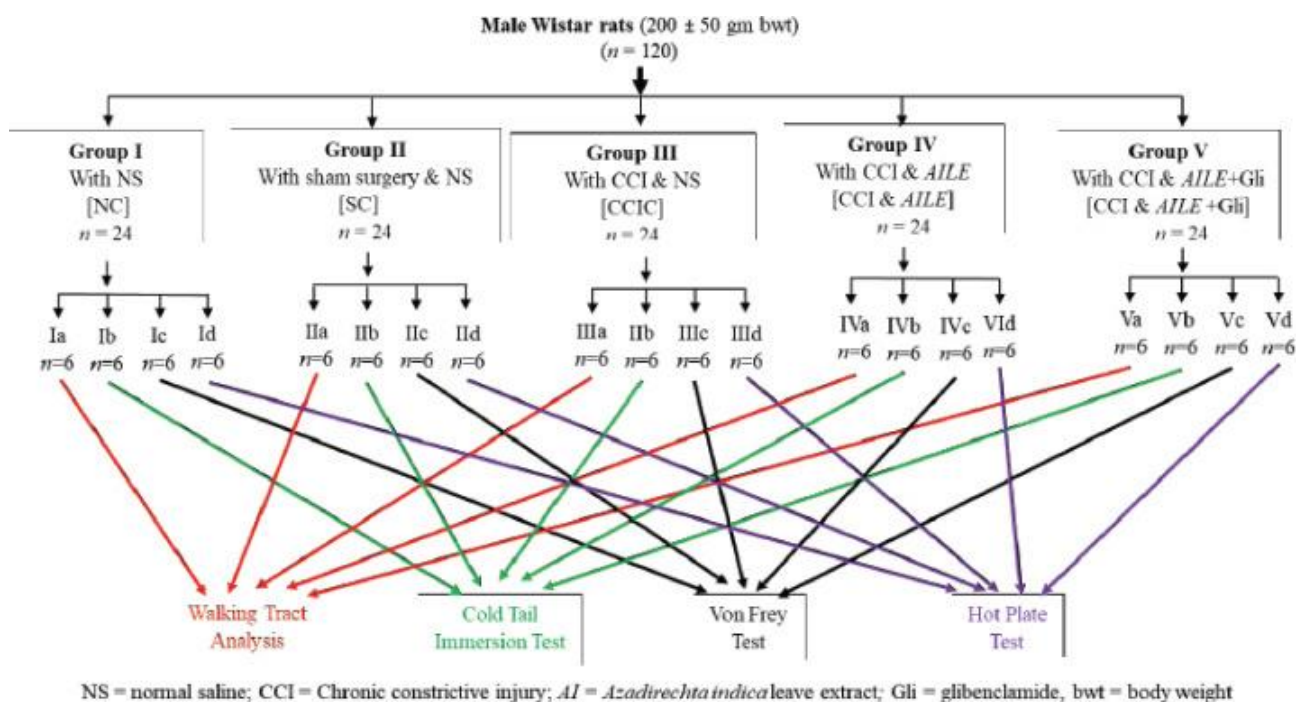
Effects of *Azadirachta indica* on neuropathic pain induced by chronic constriction injury to sciatic nerve of Wistar rat

Biswas P, Shahnaz M, Akhter M, Ripa AP, Ali T, Rafiq K.

J Adv Vet Anim Res. 2022 Sep 29;9(3):359-368. doi: 10.5455/javar.2022.i603. eCollection 2022 Sep. PMID: 36382046

Objective: The research was designed to assess the consequences of *Azadirachta indica* aqueous leaf extract (AILE) on neuropathic pain in Wistar rats and the role of the ATP-dependent potassium channel (K_{ATP}) as an underlying mechanism. **Materials and methods:** This experimental layout was conducted on Wistar rats ($n = 120$) having 150 to 200 gm of body weight. On the foundation of the experimental design, rats were divided into group I (normal saline, 5 ml/kg/body weight) and group II (sham surgery and treatment with NS), group III [chronic constriction injury (CCI) in the sciatic nerve; and treated with NS], group IV (CCI and treated with AILE 400 mg/kg body weight), Group V (CCI, pretreated with Glibenclamide 15 mg/kg followed by treated with AILE 400 mg/kg). All the treatments were given once daily for a consecutive 21 days via the oral route, except Glibenclamide. Glibenclamide was given once through the intraperitoneal route on the day of the

experiment. **Results:** Based on the neuropathic pain evaluation test, all groups were again sub-divided into subgroup "a" (walking tract analysis), "b" (cold tail immersion test), "c" (Von Frey test), and "d" (hot plate test). AILE showed a significantly higher sciatic functional index ($p < 0.05$) in walking track analysis, tail flick latency ($p \leq 0.05$) in the cold tail immersion test, and paw withdrawal threshold ($p \leq 0.05$) in the Von Frey test compared to CCI control. In addition, a nonsignificant difference in all these above-mentioned variables between the rats with CCI plus AILE and the CCI plus AILE plus glibenclamide group indicated that the K_{ATP} channel was not involved in the beneficial analgesic effects of AILE. **Conclusions:** The outcome of the present study indicates that AILE prevented worsening of neuropathic pain after chronic constriction injury in the sciatic nerve of Wistar rats in which the K_{ATP} channel was not involved.



Effect of Purple Neem Foliage as a Feed Supplement on Nutrient Apparent Digestibility, Nitrogen Utilization, Rumen Fermentation, Microbial Population, Plasma Antioxidants, Meat Quality and Fatty Acid Profile of Goats.

Taethaisong N, Paengkoum S, Nakharuthai C, Onjai-Uea N, Thongpea S, Sinpru B, Surakhunthod J, Meethip W, Paengkoum P.

Animals (Basel). 2022 Oct 30;12(21):2985. doi: 10.3390/ani12212985. PMID: 36359109

The purpose of this experiment was to investigate the effect of Purple Neem foliage as a feed supplement on nutrient apparent digestibility, nitrogen utilization, rumen fermentation, microbial population, plasma antioxidants, meat quality and fatty acid profile of goats. Eighteen Boer male goats (approximately 20 ± 2 kg body weight; mean ± standard deviation (SD)) were randomly allocated into three treatments. All goats were fed a 60 d daily feeding with three treatments: (1) control, (2) 3% Purple Neem foliage (PNF) + 3% sunflower oil (SFO) in concentrate, and (3) 6% Purple Neem foliage (PNF) + 3% sunflower oil (SFO) in concentrate. The findings indicate that goat feed containing 6% PNF + 3% SFO in

concentrate increased feed consumption, nutrient intake, nutrient apparent digestibility and nitrogen utilization compared to the goat feed at 3% PNF + 3% SFO and the control group. The feeding of goats with 6% PNF + 3% SFO in concentrate resulted in high ammonia nitrogen, BUN, acetic acid, propionic acid, butyric acid, and the total VFA levels were increased at 2 and 4 h after feeding ($p < 0.01$). The individual microbial population with 6% PNF + 3% SFO had higher ($p < 0.01$) total bacteria, higher *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefacies*, and *Streptococcus bovis*, decreased protozoa and methanogen levels at 2 and 4 h after feeding. The antioxidant in plasma indices varied, with 6% PNF + 3% SFO having higher total antioxidant (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and catalase (CAT) antioxidant activity and lower malondialdehyde (MDA) in plasma at 2 and 4 h after feeding. Additionally, goat fed 6% PNF + 3% SFO can improve meat quality by lowering drip loss, cooking loss, shear force, and saturated fatty acid as well as increase the fatty acid profile (monounsaturated and polyunsaturated fatty acids) in goat meat. Our findings suggest that Purple Neem foliage might be an excellent alternative additive for goat feed.