https://doi.org/10.15255/KUI.2024.009
KUI-38/2024
Review
Received March 21, 2024
Accepted June 2, 2024

Neem Oil: Classification and Environmental Risk Analysis for Application in the Fertiliser Industry and Agriculture

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Abstract

Sustainable agricultural practices are increasingly important to meet the food demands of the growing world population. The most important aspect of sustainable agriculture involves gradually abandoning synthetic and harmful pesticides, and switching to natural and soil-friendly practices. Neem oil, derived from the seeds of the neem tree primarily found in India, contains the active ingredient azadirachtin. Azadirachtin serves as a pesticide against harmful insects, especially in agriculture. Its widespread utilisation stems from its benign impact on living organisms, including humans, animals and plants. This review discusses the effects of neem oil on living organisms in soil, followed by the discussion of both qualitative and quantitative analytical techniques for measuring neem oil. Additionally, the environmental effects of neem oil in agriculture are discussed in relation to the current regulations and initiatives by environmental protection organisations.

Keywords

Neem oil, nitrogenous fertiliser, coating agent, azadirachtin, fatty acids

1 Introduction

The neem tree, indigenous to tropical or subtropical regions across Asia, Africa, America, and Australia, is known for the pesticidal and nitrification inhibition properties of its seed oil.[1] The nitrification inhibition effect of neem was initially reported in the early 1970s, demonstrating its role in increasing urea-N uptake in rice cultivation.[2] Neem cake, a by-product, was utilised in studies to coat urea fertiliser granules until 1990.

Neem oil is extracted through cold pressing of neem seeds, followed by the extraction process via organic solvents.[3] The quality and composition of neem oil vary depending on the source of the raw material and the oil extraction process. For instance, the triterpenoid concentration in neem oil varies with geographical location and the season of neem seed harvest.[3,4]

The variety of health benefits of neem make it versatile for antioxidant, antifungal, antibacterial, and insecticidal applications.[5]

Despite its proven agricultural benefits[5], further research is needed to assess its long-term effects on soil flora, groundwater, and human health.

The Organic Materials Review Institute (OMRI), a non-profit organisation responsible for verifying the conformity of organic materials used in different industries with the standards of the United States Department of Agriculture (USDA), has certified Neelcoat NML as an organic product.[6] Neem oil and its derivatives are categorised as "non-synthetic (natural)" under the USDA NOP (National Organic Program) 5034-1 standard, permitting their use in "herbal fertilisers, soil enhancement, crop management, and manufacturing aids" as listed in the "Generic Materials List" of OMRI in 2022.

The effects of neem and derivatives usage in agricultural applications are reviewed in *'Hazards Associated With Neem-based Pesticide Use/ Environmental Hazards'* chapter of the Neem Fact sheet document of the United States Agency for International Development (USAID).[7] The document states that neem is biologically degradable, and that azadirachtin rapidly decomposes in soil, water, and on plants. Neem oil is easily washed away by irrigation or rain, preventing prolonged persistence, accumulation, or effective soil penetration.

Applications of neem products may need to be repeated due to its vulnerability to UV light degradation, and its thermo-sensitivity, decomposing above 50 °C. Residues from neem oil extraction can be used as fertiliser and soil enhancer.

Neem oil is a product for organic farming approved by organisations in both Europe and the US. As the current regulations and standards in the fertiliser industry do not specify a section for herbal extracts or plant-based materials usage in agriculture, this review briefly discusses the classification of neem oil in international standards and regulations, along with an assessment of the environmental risks post-application in agriculture and the fertiliser industry.

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2 Qualitative and quantitative analysis methods of neem oil

Quality analysis of herbal/vegetable oils typically involves gas chromatography-mass spectrometry (GC/MS) to evaluate fatty acid concentrations. *Gosse et al.* conducted a gas chromatography analysis of neem oil in 2005 (Table 1).[5]

Table 1 – GC analysis of neem oil

Time / min	Concentration / %	Fatty acids
6.81	17.6	Linoleic acid
7.24	0.2	Margaric acid
7.34	0.2	Behenic acid
11.02	18.6	Palmitic acid
11.82	43.1	Oleic acid
13.08	17.7	Stearic acid
15.38	0.6	Odeolidic acid
17.78	1.4	Arachidic acid
18.3	0.1	Gadoleic acid
23.62	0.3	α-Linoleic acid
32.9	0.2	Lignoceric acid

GC/MS should not be the sole method for characterising neem products. Chromatographic analysis methods, such as HPLC, are also applied to detect the azadirachtin content (CAS No: 11141-17-6) as a characteristic of neem oil. However, the IS 14300:1995 standard defines the chromatographic analysis method for determining azadirachtin content in neem products marketed in India, where neem oil coating is mandatory for urea fertilisers. This method involves extracting azadirachtin content from neem products using a methanol-water solution and analysing the solution by high performance liquid chromatography. Additionally, the AOAC 2007.001 method, a standard for pesticide residue analysis in foods, defines the chromatographic method for analysing pesticide active ingredients.

3 Evaluation of the environmental effects of neem oil

The application of neem oil in agricultural areas can lead to environmental pollution in soil, air, and water. Various studies have aimed to address this issue, examining the pollution it causes in the atmospheric environment, soil, air, and water. As environmental concerns grow, the effects of market-introduced products on greenhouse gas emissions, the circular economy, agricultural productivity of soil, water, and air pollution are being scrutinised. The long-term effects of neem oil remain uncertain, raising questions about its environmental impact.

3.1 Effects on soil

Data from neem oil application to soil have yielded various results regarding its toxic properties. However, the Environmental Protection Agency (EPA) has concluded that cold-presses neem oil, as a neem oil extract, is neither a mutagen nor a toxic substance to soil development. Based on the review and analysis of the guideline studies, it is stated that no additional toxicity data is needed to support the use of this biochemical in foods. Moreover, humans are already exposed to neem components through medicinal and cosmetic products via dermal, oral, and inhalation routes.[8]

In 2011, *Muangphra and Gooneratne* examined the toxic effects of neem extracts on earthworms.[9] Their study found a decrease in the radial thickness of the epidermis and body walls of earthworms, but no DNA structure deterioration. These effects were observed only at concentrations 25 times higher than the typical neem dose used as an insecticide in agriculture. The study reported that neem extracts exhibit cellular toxicity but not genetic toxicity, and that using neem products in the recommended limited doses poses no risk to earthworms.

In 2018, *Zortea et al.* studied the toxic effects of neem cake and neem extract on soil organisms.[10] Their data indicated that neem extract was not toxic to soil organisms up to 1000 ppm, although deformities were detected in *Enchytraeus crypticus* at concentrations above 1000 ppm.

Various studies exist on the possible toxic effects of neem oil. One such study was conducted by comparing neem oil with moringa oil, a plant species having similar chemical properties Apart from azadrachtin, which is the active ingredient of neem oil extract, moringa oil also contains organic structures such as tannin, saponin, and phytate. With the effect of these structures, they found that moringa oil was more effective than neem oil in removing toxic structures such as petroleum hydrocarbons. This study showed that agricultural materials like moringa and neem can be used in the bioremediation of soils contaminated with crude oil.[11]

Another concern regarding soil contamination is the presence of heavy metals, which can leach into and contaminate groundwater sources. In 2019, *Abu-Abdoun and Al-Balawna* conducted a study on various parts of the neem tree to examine their heavy metal content.[12] Heavy metal content is a significant restriction in the use of neem oil extraction due to regulatory and human health considerations. The study analysed the heavy metal content in different parts of the neem tree, such as the leaves, trunk, and resin, and found that the leaves contained higher levels of heavy metal compared to the trunk and resin. However, when the total heavy metal concentrations in the neem tree were examined (Table 2), these values were within the limits set by the World Health Organization (WHO) (for example, max. 10 $mg\,kg^{-1}$ for Pb).[13]

The degradation process of neem oil in soil varies according to the differences in the chemical structure of the azadirachtin it contains. In nee m oil, azadirachtin exists in two different chain structures classified as A and B, and

Table 2 – Heavy metal concentrations in different parts of the neem tree[12]

Neem type	Fe	Mn	Co	Ni	Cu	Zn	Cr	Cd	Pb	Mo	As	B	Ca
	ppm												
Neem wood 1	158.66	7.20	0.16	1.74	7.46	3.48	0.61	0.00	0.63	0.00	0.00	28.85	8611.59
Neem wood 2	131.14	6.04	0.14	1.53	6.57	2.85	0.44	0.01	0.58	0.00	0.00	28.21	7575.47
Neem wood 3	148.66	16.67	0.11	1.42	13.66	7.08	0.81	0.11	0.49	2.97	4.52	23.48	–
Neem gum 1	131.61	3.99	0.10	1.20	9.38	2.98	0.92	0.01	0.42	2.43	4.75	4.16	–
Neem gum 2	92.81	2.68	0.08	0.88	4.96	3.66	0.27	0.01	0.26	0.00	0.00	3.31	7667.00
Neem leaf 1	79.71	7.16	0.61	0.61	BDL	5.10	0.18	0.01	0.25	0.00	0.00	121.15	11188.15
Neem leaf 2	171.37	9.87	1.58	1.58	2.42	8.68	0.45	0.01	0.61	0.00	0.00	117.60	19118.16
Neem leaf 3	329.87	15.41	2.40	2.40	5.32	13.44	1.33	0.01	0.79	1.89	5.54	217.91	–

these structures follow different degradation processes in water and soil.[14] When examining the degradation process of cold-pressed neem oil in soil, *Sundaram et. al.*[15] reported that azadirachtin A limonoid contained in neem oil persists for 3–6 days on terrestrial matrices and 8–13 days in water. While the half-life of azadirachtin B is generally shorter, the half-life of azadirachtin limonoids depends on factors such as light, temperature, and soil structure.

In summary, based on the studies[8–15] previously mentioned regarding the possible toxicity effects of neem products, their agricultural application appears to be safe for the environment. However, current agricultural and fertiliser regulations do not specifically define neem products or any other herbal products.

According to the document published by the EPA regarding the application of cold-pressed neem oils as pesticides, no toxicity risk was found for soil creatures such as birds, plants, earthworms, and non-target organisms (Annex 7, Annex 8).[8] Although neem oil was found to be slightly toxic to aquatic organisms, the Risk Quotients (RQs) were calculated to be below the Levels of Concern (LOCs) for threatened and endangered species (approximately 500 $\mu l\ l^{-1}$)[13](Table 3).[8]

Table 3 – Estimated cold pressed neem oil residues in terrestrial matrices using Terrestrial exposure model (T-Rex; EPA, 2005)[8]

Terrestrial matrix	Dietary-based estimated environmental concentrations / ppm		
	0 days after last application	86 days after application	106 days after application
Edible broadleaf plant foliage	881.20	0.04	0.00
Fruits, pods, and seeds	97.91	0.00	0.00

3.2 Effects on water

Water pollution is the basic criterion when evaluating the risks of novel agricultural applications. Nitrate leaching from agricultural soil is one of the most dominant factors affecting groundwater and drinking water quality.

In 2022, *Sriraj et al.*[16] studied the effects of neem oil extract on soil properties, growth, yield, and inorganic nitrogen content of lettuce. They observed that neem extract applications significantly reduced nitrate concentration in the soil. These results indicate that neem extracts may play an effective role in reducing nitrate leaching from soil into groundwater.

In 2022, *Abeka et al.* examined the effectiveness of neem oil and biochar in reducing nitrate leaching in composts treated with Ferric Luvisol as a nitrification inhibitor. Neem oil can also be used in soil applications to prevent nitrate leaching. In one such study, a neem oil extract with an azadrachtin concentration of 3.92 $mg\ g^{-1}$ was reported to have soil-improving properties, inhibiting nitrate leaching in soil by 40 %. The effect of azadirachtin in neem oil on the nitrification process, alongside its use as a pesticide, is crucial for agricultural sustainability. Research[17] has shown that the amount of azadirachtin in neem oil is vital for controlling the nitrification process in soil, with increased azadirachtin levels leading to a more effective nitrification process.

A 2016 study conducted by *Adamu et al.* investigated the production of biodegradable grease from neem seed oil and its characterisation.[18] The biochemical oxygen demand (BOD) test results were within acceptable limits of 4–8 ppm. Comparative BOD tests with a commercial grease product showed that grease derived from neem oil had higher BOD values, indicating its biodegradability (Table 4).

Table 4 – Biochemical oxygen demand test results of commercial and neem-based biodegradable grease samples[18]

Grease sample	Amount of oxygen after 2 h	Amount of oxygen after 5 h	Biochemical oxygen demand (BOD)
	ppm		
Ammagrease (control)	7.60	7.49	6.0
Sample 1	7.0	6.3	21.0
Sample 2	6.7	6.2	15.0

International regulations applied in the US and EU countries impose no specific limitations on herbal oils. According to Turkey's Water Pollution Control Regulation, the upper limits for oil content in water are set for different classes of intra-continental water resources as: 0.02, 0.3, 0.5, and > 0.5 ppm for Class I, II, III, and IV water qualities, respectively.

In the document published by the US EPA regarding the application of cold-pressed neem oils as pesticides, the risks of mixing neem oil with drinking water were evaluated. They report that there is no significant risk of contamination or residue accumulation in drinking water, and that health risks are minimal if neem oil is accidentally mixed with drinking water. The document also states that neem oil does not harm the health of babies and children provided the recommended doses are not exceeded.

The maximum allowable concentration of azadirachtin in neem oil is 4000 ppm, and the maximum allowable amount of azadirachtin in case of mixing with drinking water after any application is 0.1 $\mu g\,l^{-1}$.[19]

4 Application type and rates of neem oil in agricultural products

The Ministry of Fertilisers and Chemicals of India specifies that the neem oil rate extractable in benzene on urea fertilisers must be at least 0.035 % (350 ppm).

The maximum application limit for 100 % cold-pressed neem oil for agricultural use, as accepted by the US EPA, is 0.47 $l\,da^{-1}$ (1/2 $gal\,ac^{-1}$) in fields. The neem oil coating rate applied on granular urea fertilisers typically ranges from 0.1 to 0.8 %. Due to the slow-release feature of the product, the recommended application dose for neem oil coated urea fertiliser is generally 30 $kg\,da^{-1}$ (75 % of the normal fertiliser dose) for wheat production, compared to 40 $kg\,da^{-1}$ for conventional/normal urea fertiliser.

Even when the maximum rate of 0.8 % neem oil is applied to coat urea fertilisers, the rate of neem oil applied to the field would be 0.96 ppm (960 ppb), which is well below the EPA limitation, assuming the application rate of urea fertiliser of 30 $kg\,da^{-1}$ and the soil amount in the field of 250 $tons\,da^{-1}$.

Although studies on the agricultural application of neem oil are not comprehensive enough to draw definitive conclusions about its environmental effects, the results of the studies[8–15] on other more concentrated neem products, like neem cake and neem extracts, indicate low toxicity levels of neem oil products containing azadirachtin.

5 Evaluation of the risks to human health and the environment

To evaluate the effects of neem oil on human health as a result of its effects on soil, the most commonly used type is "cold-pressed neem oil". Cold-pressed neem oil has been used for hundreds of years to control plant insects and diseases.[12] Table 5 lists various toxic effects of cold-pressed neem oil on human health.

No risks to human health are expected from the use of cold-pressed neem oil due to its low toxicity across all routes of exposure.

6 Conclusion

Neem oil can enhance agricultural production yield and crop quality due to its nitrification inhibition and pesticide effects. Cold-pressed neem oil, in particular, is an environmentally friendly choice over other chemical nitrification inhibitors and pesticides.

By enhancing nitrogen use efficiency, neem oil not only promotes higher yield and better quality of the crops and grains but also allows for reduced fertiliser application. This reduction aligns with the goals of the European Green Deal Strategies and benefits farmers by enhancing agricultural yield while lowering fertiliser costs. Although this study discusses the effects of neem oil on soil and other agricultural parameters, the neem oil compositions mentioned have no direct relationship with any commercial chemical composition. In this respect, all neem oil compositions mentioned in the literature actually reveal the positive results of neem oil compositions applied to soil.

In the production of neem oil, depending on the type and amount of heavy metals it contains, no toxic structures have been encountered both during commercial-scale production and standardisation processes under laboratory conditions. The absence of negative examples in the literature does not pose a problem for the use of neem oil and its derivatives in the agricultural industry.

ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge the Toros Agri Industry and Trade Co. Inc. for financially supporting this study.

Table 5 – Human toxicology data requirements for cold-pressed neem oil[8]

Components Biochemical Data request according to OCSPP Guide Number	Office of Chemical Safety and Pollution Prevention (OCSPP) guideline number	Description of the results	Toxicity category
Oral toxic limit observed in rats	870.1100	Minimum 5000 mg kg^{-1} for female rats	IV
Form of poisoning through the skin in rabbits	870.1200	Minimum 2000 mg kg^{-1} for male and female rabbits	III
Inhalation poisoning in rats	870.1300	Toxic limit for male and female rats varies between 2.11 mg l^{-1} and 2.53 mg l^{-1}, depending on the 0.3 % and 0.15 % concentrations of azadirachtin	IV
Eye irritation in rabbits	870.2400	No eye irritations in rabbits have been reported.	IV
Skin irritation in rabbits	870.2500	No skin irritations in rabbits have been reported	IV
Skin sensitivity for guinea pigs	870.2600	No symptoms were observed during the 24-hour and 48-hour periods	No skin sensitivity has been observed
Oral feeding of mice was monitored for 90 days	870.3100	No problems were observed at maximum 5000 mg kg^{-1}	No sub-chronic oral toxicity was observed
A 90-day respiratory study was conducted	870.3250	The product is not intended for application to human skin. Therefore, no oral or dermatological toxicity has been reported in the literature.	No sub-chronic dermal toxicity was observed
A 90-day respiratory study was conducted	870.5100	No problems were reported in repeat tests where the respiratory tract was exposed in 4-hour periods	No sub-chronic inhalation toxicity was observed
Research study on poisoning of both respiratory and reproductive systems of rats	870.3500	No negative effects on the respiratory and reproductive activities of rats were reported	It is declared that as long as approved laboratory studies are followed, no problems will occur
Mutagenicity test	870.5100	There is no evidence of mutagenicity	It is not considered a mutagen

List of abbreviations

OMRI – Organic Materials Review Institute
USDA – United States Department of Agriculture
NOP – National Organic Program
USAID – United States Agency for International Development
GC/MS – Gas Chromatography/Mass Spectrometry
HPLC – High Performance Liquid Chromatography
EPA – Environmental Protection Agency
WHO – World Health Organization
BOD – Biological Oxygen Demand
BDL – bellow detectable limit

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SAŽETAK

Ulje nima: klasifikacija i analiza ekološkog rizika primjene u industriji gnojiva i poljoprivredi

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Održive poljoprivredne prakse sve su važnije za zadovoljavanje prehrambenih potreba rastuće svjetske populacije. Ključni aspekt održive poljoprivrede uključuje postupno napuštanje sintetskih i štetnih pesticida te prijelaz na prirodne, ekološki prihvatljive metode. Ulje biljke nima, koja primarno raste u Indiji, dobiva se iz sjemenki biljke. To ulje sadrži azadirahtin, pesticid učinkovit protiv insekata, osobito u poljoprivredi. Njegova široka primjena proizašla je iz benignog učinka na žive organizme, uključujući ljude, životinje, okoliš i biljke. Ovaj pregledni rad analizira učinke ulja nima na organizme u tlu, a zatim se fokusira na kvalitativne i kvantitativne tehnike za analizu ulja nima. Također su komentirani ekološki učinci ulja nima u poljoprivredi u kontekstu postojećih regulativa i inicijativa organizacija za zaštitu okoliša.

Ključne riječi
Ulje nima, dušično gnojivo, sredstvo za oblaganje, azadirahtin, masne kiseline

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Pregledni rad
Prispjelo 21. ožujka 2024.
Prihvaćeno 2. lipnja 2024.