
# Green Pesticides Handbook
# Essential Oils for Pest Control

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## Neem Oil

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# 20

# Neem Oil

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**CONTENTS**



## 20.1 Botany

### 20.1.1 Origin and Distribution

Neem, botanically known as *Azadirachta indica* A. Juss, belongs to the Meliaceae (mahogany) family (Anon, 2011). It is indigenous to India and found in tropical and subtropical regions like Pakistan, Bangladesh, Sri Lanka, and Mayanmar. The Siwalik hills, dry forests of Andhra Pradesh, Karnataka, and Tamil Nadu (India) are the main habitat of the wild population (Hashmat et al., 2012). It thrives well in the dry regions of the northwest, and approximately 50% of the tree population of India is reported in Uttar Pradesh. The Science and Technology Panel of the International Development National Research

Council (1992) has documented that around 60% of the total neem population of the world inhabits India (Tinghui et al., 2001). It is also grown and naturalized in Southeast Asian (Thailand, Indonesia, Peninsular Malaysia, the Philippines, and Singapore) and West African countries, as well as Australia and Saudi Arabia. More recently, it has been familiarized to the Caribbean and various zones of America (Parotta, 2001).

### 20.1.2 Taxonomical Classification

The neem plant is taxonomically classified as (Girish and Shankara, 2008; Anon, 2011)

Kingdom: Plantae
Division: Tracheophyta
Class: Magnoliopsida
Order: Sapindles
Family: Meliaceae
Subfamily: Melioideae
Tribe: Melieae
Genus: *Azadirachta*
Species: *indica*

*A. indica* is synonymous with *Melia azadirachta* L. and *Antelaea azadirachta* (L.) Adelb (Anon, 2011).

### 20.1.3 Vernacular Names

*A. indica* has many local names, depending on the languages used in a country. The appellations given in the different languages of India and other countries are shown in Table 20.1.

### 20.1.4 Plant Description

*A. indica* belongs to Meliaceae, a family of dicots mostly represented by trees and shrubs. The family includes about 51 genera and 550 species, with many of them prized for their wood, edible fruits, and medicinal and ornamental qualities (Wiart, 2006). It is a small to medium-sized evergreen tree with a height of 15 m (30 m maximum), having a large rounded crown (10–20 m) with spreading branches and a branchless bole (7.5 m, diameter 90 cm). The bark of the tree is thick, fissured, dark gray to red (inside) in color, and it possesses a gummy colorless sap. The leaves are long (20–40 cm), alternate, pinnate, exstipulate, and glabrous with a light green hue. The leaves have two pairs of basal glands with a subglabrous petiole (2–7 cm) and above, channeled rachis. Each leaf comprises 8–19 serrated, proximally alternate, ovate to lanceolate leaflets. Inflorescence is axillary clustered multiflowered thyrsus (150–250 flowers) with a length of 15–30 cm and minute caducous bracts. Flowers of the tree are small (1 cm in diameter), white or pale yellow, and sweet smelling. They are actinomorphic, pentamerous, and bisexual or unisexual male on the same plant. The calyx of the flowers is imbricate, ovate, thin, and puberulous from inside, while petals are free, spreading, imbricate, spathulate and ciliolate from inside. Fruits are single (maximum of two) and small (1–2 cm) in size. They are greenish to yellow in color and an ellipsoidal seeded drupe. The tree has a thin exocarp, pulpy mesocarp,

**TABLE 20.1**

Vernacular Names of *A. indica*

| Country | Language: Vernacular Names |
|---|---|
| India | Bengali: Nim, nimgachh<br>Guajarati: Danujhada, limbado, limbra, limdo<br>Hindi: Balnimb, neem, nim, nimb, nind, vempu, veppam<br>Kannada: Bemu, bevinamara, bivu, kaybevu<br>Punjabi: Bakam, drekh, nim<br>Sanskrit: Arista, nimba, nimbah, picumarda<br>Tamil: Vepa, veppu, veppam, vembu |
| Indonesia | Indonesian: Mind, intaran, membha, imba, mempheuh, mimba<br>Javanese: Mimba, imba |
| Malaysia | Malay: Sadu, baypay, mambu, veppam |
| Myanmar | Burmese: Bowtamaka, thinboro, tamarkha, tamar, tamaka, tamabin |
| Nepal | Nepali: Neem |
| Thailand | Thai: Sadao, kadao, sadao India, khwinin, saliam, cha-tang |
| Vietnam | Vietnamese: Saafu daau, sàu-dàu |
| England, Canada, America | English: Persian lilac, neem tree, bastard tree, Indian lilac, bead tree, margosa tree, cornucopia, Indian cedar |
| France | French: Margousier, margosier, neem, nim, azadirac de l'Inde |
| Saudi Arabia | Arabic: Nim, neem |

*Source:* Parotta, J. A., *Healing Plants of Peninsular India*, CABI Publishing, New York, 2001, pp. 495–496.

and cartilaginous endocarp. Seeds are an unwinged, oval, or spherical structure with thin testa. The tree has a profound taproot system with widespread lateral roots. It may form suckers if roots encounter some damage (Hearne 1975; Csurhes, 2008; Hashmat et al., 2012).

## 20.2 Methods of Extraction of Oil

Neem essential oil is usually prepared from the seed kernels and is well known for its high insecticidal and medicinal value (Lokanadhan et al., 2012). The fruit, flower, and leaves are minor sources of neem essential oil (Narsing Rao et al., 2014). According to a survey, only 20% of the seeds are being harvested due to scattered growth of neem trees in India. Out of it, India produces approximately 8300 tonnes of neem oil annually. In general, the neem oil yield reported from seeds varies from 25% to 45% (Anya et al., 2012; Ismadji et al., 2012). Mechanical pressing, solvent extraction, and more recently, supercritical fluid extraction (SFE) are among the numerous methods to extract neem seed oil (NSO) (Liauw et al., 2008). In the mechanical method, cold pressing or temperature-controlled pressing can be employed to procure oil from the neem seed kernel via physical crushing. Approximately 82% of the neem oil can be recovered by the mechanical extraction method. Although mechanical pressing is a frequently used technique, neem oil acquired with this process has poor quality due to it low azadirachtin content (1427 ± 51 ppm, 25.3%), suggesting nonselectivity of the extraction process. Moreover, oil produced by mechanical pressing is turbid, containing a considerable amount of water and metal content, and hence it has cheap market value (Adeeko and Ajibola, 1990; Lalea and Abdulrahman, 1999). According to a study conducted by Nitièma-Yefanova et al. (2012), the oil yield by cold pressing has

a positive effect on increased kernel compression, reduced particle size, and decreased cage loading. It was concluded that the best oil yield (40.3% ± 0.0) from ground kernels can be obtained by cold pressing at 25°C, 33.7 MPa ± 2.9 pressure, and quarter-cage loading. However, while the cold-pressing method is easy, economical, and solvent-free, to obtain high-quality oil, intensive purification steps are needed, which often reduce active components of oil and may cause technical and monetary constraints for commercial-scale production (Ismadji et al., 2012).

The solvent extraction method is generally the preferred choice for obtaining neem oil. It furnishes a high yield and clear oil compared with the mechanical extraction method. In the Soxhlet extraction method, neem oil percentage recovery corresponded to 92.3%–99.1%, with the azadirachtin content (4658.4 ± 92.5 ppm) three times more than that obtained from the cold-pressed extraction procedure. Furthermore, the conventional solvent extraction method has a relatively low operational cost and is economical compared with the other modern methods of extraction, like supercritical fluid extraction (Liauw et al., 2008; Adewoye and Ogunleye, 2012; Ismadji et al., 2012). Hexane is the most commonly used solvent in the extraction of seed oil, including neem seeds, due to its suitable functional properties, that is, nonpolar nature, which facilitate the high solubility of hydrocarbons, lipids, and glycerides at moderate temperatures. Also, hexane is inexpensive and unreactive with oil (Ayoola et al., 2014). However, it is enlisted among 189 hazardous air pollutants of the Clean Air Act, and is being watched as both a "criteria pollutant" and a "hazardous air pollutant." Thus, interest has been generated among researchers to discover an alternative, nonflammable, efficient, less hazardous, and environmentally friendly solvent. Liauw et al. (2008) compared *n*-hexane and ethanol solvents as a medium of neem oil extraction. The maximum oil yields obtained were 41.11% and 44.29% with ethanol and *n*-hexane at a low extraction temperature (50°C), respectively. At the same temperature, petroleum ether (42.60%) demonstrated higher efficiency than ethanol (39.17%) for neem oil extraction (Satyanandam et al., 2011). Later, it was anticipated that ethanol may be a good substitute for hexane, as there is a possibility to enhance oil extractability using ethanol at high temperatures (Liauw et al., 2008; Ayoola et al., 2014). This can be easily achieved because of ethanol's tendency to withstand high temperatures (80°C), which is not possible in the case of hexane due to its highly flammable behavior. Also, there were several reports that showed that ethanol–hexane mixtures have the potential for high neem seed oil extraction compared with hexane alone. In one study, ethanol–hexane mixtures utilized for oil extraction by the Soxhlet extraction method in ratios of 60/40, 50/50 and 40/60 (v/v) furnished oil yields of 44%, 43%, and 41.2%, respectively. The ethanol–hexane mixtures gave a better yield than 100% hexane (40.25%) at 55°C over a 6 h duration (Ayoola et al., 2014). Similarly, Edres (2014) extracted neem oil through the Soxhlet extraction method using only *n*-hexane and obtained a very low yield of 17.60%.

The high oil yield by the solvent extraction method depends not only on the type of solvent, but also on various other physical factors, such as solvent composition, volume of solvent, temperature, sample–solvent ratio, and material size. Liauw et al. (2008) has optimized oil extraction from ground neem seeds, categorized into three types of particle size ranges (i.e., 0.85–1.40 mm, 0.71–0.85 mm, and 0.425–0.71 mm). The results revealed that the maximum oil yield was obtained at 0.425–0.71 mm seed sizes. Adewoye and Ogunleye (2012) improved neem oil extraction with regard to the three aspects of extraction, that is, solvent composition (*n*-hexane and ethanol), temperature, and time, by a central composite design (CCD). The maximum predicted percentage yield was obtained as 43.48%

with 80.77% *n*-hexane at 34.93°C over a 6 h duration. Okonkwo et al. (2013) reported the influence of agitation, type of impeller, and time of contact on NSO yield using a pilot solvent extraction plant. In their research, the maximum percentage yield was found to be 36.86% within 40 min with a flat-blade turbine impeller type A1 operating at 84 rpm. At 50°C, food-grade ethanol was used as a medium for extraction with 0.425–0.710 mm seed particle size.

Among solvent extraction procedures, the Soxhlet method is conventionally performed for oil extraction (Ayoola et al., 2014). Because of its low yield and to meet demand, recently, microwave-assisted extraction (MAE) was manipulated for optimization of neem oil extraction by Doehlert design. Parameters such as time, temperature, and solvent-to-biomass ratio were studied to obtain an optimized yield of neem oil (Nde et al., 2015). It was observed that within 24 min, 80% of the oil was extracted at 80°C and a solvent–biomass ratio of 3:1. This method was found to be more efficient and faster than the conventional method (10 h), and without significantly affecting oil quality. This attributed to the penetrative behavior of microwaves, as shown by scanning electron microscopy (SEM) analysis, which revealed structural deterioration of the neem seed kernels.

Furthermore, SFE has been of great interest for being an efficient and effective method for the recovery of essential oils and active metabolites from solid matrices. It produces high-quality oil with no solvent residues, but its operating and investment costs are high. Beyond a critical temperature (31°C) and pressure (74 bars), carbon dioxide ($CO_2$) exists as a liquid and finds use in SFE to extract the active ingredients of neem seeds (Martinelli et al., 1991). The density and solvation of the supercritical $CO_2$ (SC-$CO_2$) at 200 bars is analogous to that of hexane; hence, it behaves as a nonpolar solvent and can dissolve triglycerides at concentrations up to 1% mass. Unlike other solvents, $CO_2$, being a green, nonhazardous, inert, and affordable solvent, is perfectly adapted to extract essential oils without interfering with its active components (Sapkale et al., 2010). Contrary to the solvent extraction method, SFE is environmentally friendly and produces no waste, and removal of solvent (SC-$CO_2$) from the oil or analyte does not require rigorous heating, which can be achieved by releasing pressure, leaving almost no trace of $CO_2$ in the oil yield. By using SC-$CO_2$, extraction of neem essential oil and its active triterpenoids has been reported elsewhere (Mongkholkhajornsilp et al., 2005; Zahedi et al., 2010).

The solvation properties of SC-$CO_2$ can be tailored by adding cosolvents such as ethanol and methanol. Johnson and Morgan (1997) reported SC-$CO_2$ extraction of neem seed oil and its triterpenoids (azadirachtin, nimbin, and salannin) at 328.15 K with methanol to enhance the selectivity and extraction performance. There have been studies that have suggested that the solvation properties of SC-$CO_2$ can be modulated by altering temperature and pressure as well, to obtain a high oil yield. The adjustable solvent power offers high extraction selectivity. Ismadji et al. (2012) demonstrated the extraction of neem oil and active triterpenoid compounds from seed kernel without cosolvent through changeable pressures (10–35 MPa) and temperatures (313.15, 323.15, and 333.15 K). It was observed that the neem oil yield and active molecules extracted were temperature and pressure dependent. The maximum percentage recovery of neem oil was reported as 6.67 ± 0.12 ($\times 10^3$ kg) at the highest studied temperature (333.15 K) and pressure (35 MPa). This was explained by the high pressure greatly increasing the solvent density (solvating power) of $CO_2$, thus enhancing the solubility of the solute. Moreover, the solvent-to-solute ratio ($CO_2$ to neem seed kernels) has a significant impact on the oil yield at supercritical conditions, as revealed by Ambrosino et al. (1999).

## 20.3 Methods of Analysis of Oil

Essential oils are widely used for medicinal, cosmetic, culinary, perfumery, and agricultural products. The geographical location, climatic conditions, soil properties, chemotype, developmental stages, and so forth, affect the distribution of components and manifest quantitative changes in the oil composition. However, chemical fingerprinting or key active components help to determine the purity as well as potency of the oil. Due to the high market price of premium essential oils, they are adulterated, diluted, or substituted with poor-quality oils, cheap terpenes, and low-density petroleum fractions. Therefore, analyses of oil to check adulterant and purity is of utmost importance to consumers and manufacturers for safety and quality control.

Essential oils are complex mixtures of secondary metabolites, responsible for their unique properties and aroma. The characterization of oil for their chemical composition is vital for industrial and economic purposes, where organic chemistry plays a fundamental role. The growth of chromatographic techniques has made significant progress in the study of the chemical composition of essential oils, for example, high-performance liquid chromatography (HPLC), gas chromatography (GC), gas chromatography–mass spectrometry (GC-MS), and capillary electrophoresis (Forim et al., 2010; Djenontin et al., 2012). GC is considered one of the best methods for analyzing oils, for both qualitative and quantitative determination, due to its simplicity, rapidity, and efficiency. Generally, conventional GC analyses require 30–60 m columns and 30–60 min to furnish a chromatogram. The high efficiency of chromatography and quantitative determination of the important groups of a compound were achieved by baseline separation. It is imperative that all reliable analytical methods have prior validation. The criteria for authentication usually include linearity, specificity, accuracy, precision, robustness, recovery, limits of quantification (LOQs) and detection (LODs), and repeatability in HPLC methods. Along with these chromatographic techniques, development of detectors acted as a powerful tool for accurate analyses of essential oils (Forim et al., 2012).

The interesting feature of neem essential oil is that it is potently aromatic and viscous, and predominantly constitutes fatty acids, along with hydrocarbons (Djenontin et al., 2012). In order to obtain fatty acid profile of neem oil, fatty acid methyl ester (FAME) derivatives were injected into a GC equipped with a flame ionization detector (FID) with a glycol succinate column (Hossain, 2005). For identification of individual fatty acids, both internal and external standards were used. Recently, Djenontin et al. (2012) reported the neem oil profile by GC-FID, involving both identification and quantitation of its components. Among fatty acids, prominent concentrations of oleic (43.5%), linoleic (18.7%), palmitic (17.8%), and stearic (17.4%) acids were observed. During analysis, a HP-INNO Wax capillary column and helium as the carrier gas were used. Furthermore, the composition of neem essential oil was explored by both chromatographic and spectral techniques, such as quantitative thin-layer chromatography (TLC), GC, GC-MS, and $^{13}C$ nuclear magnetic resonance (NMR). They revealed that fatty acids were the main component of NSO. Other compounds include triacylglycerols, sterols, *n*-alkanes, aromatics, esters, sulfur, and nitrogenous compounds and terpenoids (Kurose and Yatagai, 2005; Momchilova et al., 2007). The fatty acid of neem oil was also studied by high-speed countercurrent chromatography (HSCCC) (Gossé et al., 2005). They successfully resolved different fatty acids and unsaponified organic compounds via the HSCCC technique.

In neem essential oil, azadirachtin is a good-quality control biomarker compound that possibly simplifies the demand of various equipment, time, and the cost required for extensive analyses, to check the purity of the oil and its industrial formulations. The amount of azadirachtin is usually detected in various neem oil products by a simple, sensitive, and selective HPLC technique (Ambrosino et al., 1999; Sidhu et al., 2003). Forim et al. (2010) developed a method for simultaneous quantification of two important limonoids of NSO, that is, azadirachtin and 3-tigloylazadirachtol, by using HPLC equipped with an ultraviolet (UV)–visible detector. The chromatographic analyses were performed isocratically with a ratio of 35:65 (v/v) of acetonitrile and water as the mobile phase, and the UV wavelength was selected at 217 nm (maximum) to measure low-concentration marker compounds. The study was aimed at controlling the quality and promotion of Brazilian neem seed and NSO. In another study, analysis was performed by a reverse phase Spherisorb C-18 ODS 5 µm column with an acetonitrile–water gradient system to determine the azadirachtin content in neem formulations and oil (Sundaram and Curry, 1993). Similarly, a fast preconcentration method was established for azadirachtin A, azadirachtin B, nimbin, and salannin determination in neem oil. It comprises solid phase extraction with graphitized carbon, followed by quantification using HPLC-UV with an upper limit of quantification, 100 µg/ml (Ramesh and Balasubramanian, 1999).

Moreover, HPLC combined with a mass spectrometry (MS) detector has been utilized by researchers to study the azadirachtin content of neem oil extracted from insecticidal formulations (Ambrosino et al., 1999; Barrek et al., 2004). MS is a more precise tool for the identification of compounds than a conventional UV attachment. In the study, atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) were used as ionization sources, and these enabled rapid identification of neem oil limonoids. The ESI source assisted in the identification of the largest number of structures (azadirachtin A, azadirachtin D, azadirachtin I, deacetylnimbin, de-acetylsalannin, nimbin, and salannin) (Barrek et al., 2004). In another study on seed oil, HPLC analysis coupled with a UV diode array detector (DAD) indicated the presence of sterols, where the major sterol was β-sitosterol (77.7%) (Djenontin et al., 2012). Similar studies on sterols and tocopherol of NSO were carried out by GC and HPLC, respectively (Djibril et al., 2015). Although the above-discussed chromatographic techniques combined with detectors may be utilized for more precise screening, identification, and quantification of essential oils, they are expensive for regular analyses. Lalla et al. (2003) developed a cheap, simple, accurate, and specific method to analyze NSO using azadirachtin as a biomarker compound. The method employs high-performance thin-layer chromatography (HPTLC) for quantitative study of the oil and can be exploited for routine analyses. More recently, HPTLC was carried out to investigate the composition of NSOs obtained from three production sites in Italy. The study exhibited a marked difference in chemical composition and variation among NSOs, with respect to the limonoids (Benelli et al., 2015).

## 20.4 Composition of Oil

Neem essential oil from flower and leaves is a minor source of volatile oil (0.08%), composed mainly of caryophyllene (85%) (Narsing Rao et al., 2014). NSO is a main source of volatiles, being composed of essential oil and fatty acids (Djenontin et al., 2012). NSO is

subjected to extensive phytochemical studies due to its strong biological, agricultural, and medicinal properties (Lokanadhan et al., 2012). The chemical composition of NSO is very complex and rich in terpenoids, limonoids, and volatile sulfur compounds (Ricci et al., 2009). Until now, more than 300 compounds have been isolated from various parts of *A. indica* (Gossé et al., 2005). However, NSO alone recounted more than 100 determined biologically active compounds (Benelli et al., 2015). The key chemical constituents reported from neem essential oil can be divided into individual classes, as described in the next sections.

### 20.4.1 Hydrocarbons

El-Hawary et al. (2013) has studied essential oils obtained from neem leaves and flowers using the hydrodistillation method. The constituents of leaf and flower essential oil were analyzed by GC-MS. The main hydrocarbons (85.36%) detected in leaf oil were β-elemene (33.39%), γ-elemene (9.89%), germacrene D (9.72%), caryophyllene (6.8%), and bicyclogermacrene (5.23%). The oxygenated compounds were mainly sesquiterpene oxide (5.04%). However, flower oil hydrocarbons were composed primarily of pentacosane (18.58%), tetracosane (10.65%), β-germacrene (9.73%), β-caryophyllene (5.84%), and dodecene (4.54%). The principal oxygenated compounds (28.3%) were octadecanol (16.7%), verdiflorol (5.32%), farnesol (1.63%), and α-terpineol (1.51%). In addition, Narsing Rao et al. (2014) showed that volatile oil obtained from neem flower powder by hydrosteam distillation contains the sesquiterpenes caryophyllene (56.03%), caryophyllene oxide (17.41%), and α-caryophyllene (humulene) (12.10%) as major components. The other minor components were identified as copaene, bicyclo[5,2,0]non-1-ene, cyclohexene, 2H-indene 2-one, cyclohexane, and 1,2-benzenedicarboxylic acid. The neem seed essential oil has been studied by Kurose and Yatagai (2005), along with other essential oils of *Azadirachta* species. GC and GC-MS have detected hexadecanoic acid (34.0%), oleic acid (15.7%), 5,6-dihydro-2,4,6-triethyl-(4H)-1,3,5-dithiazine (11.7%), methyl oleate (3.8%), and eudesm 7(11)-en-4-ol (2.7%) as major oil constituents. The minor components spotted were *n*-alkanes, aromatics, esters, sulfur and nitrogen compounds, and terpenoids.

### 20.4.2 Fatty Acids

NSO is a major source of fatty acid and is mainly composed of oleic acid (50%–60%), palmitic acid (13%–15%), stearic acid (14%–19%), linoleic acid (8%–16%), and arachidic acid (1%–3%). Oleic acid, linoleic acid, and α-linoleic acid are the principal ω-9, ω-6, and ω-3 fatty acids, respectively, in NSO (Mongkholkhajornsilp et al., 2005; Ismadji et al., 2012). Besides, palmitic (31.76%), linoleic (18.57%), linolenic (12.64%), oleic (9.74%), arachidonic (7.38%), and docosatrienoic (5.7%) acids were also reported in flower oil (Narsing Rao et al., 2014). Nevertheless, Momchilova et al. (2007) identified and quantified 13 fatty acids and 25 triacylglycerols by amalgamation of chromatographic and spectral techniques.

### 20.4.3 Limonoids

The important bioactive compounds of NSO belong to the limonoid class of triterpenoids, such as azadirachtin (azadirachtin A), salannin, salannol, nimbin, nimbinin, nimbidin, nimbidiol, nimolicinol, gedunin, 3-tigloylazadirachtol (azadirachtin B), epoxyazadiradione, 17β-hydroxyazadiradione, 1-tigloyl-3-acetyl-11-hydroxymeliacarpin (azadirachtin D),

1α,2α-epoxy-17β-hydroxyazadiradione, 1α,2α-epoxynimolicinol, and 7-deacetylnimolicinol (Hallur et al., 2002; Ismadji et al., 2012). In 1942, Siddiqui reported bitter principles, nimbin, nimbinin, and nimbidin, where nimbidin was the major bitter principle of NSO. All these plant metabolites are well known for their effective biological properties against insects and pests; among them, azadirachtins (0.3%–0.6%) are the most active component of neem essential oil (Brahmachari, 2004). The concentration of triterpenoid secondary metabolite in neem seeds is dependent on the geographical location of the plant grown (Sidhu et al., 2003).

#### 20.4.3.1 Azadirachtins

Azadirachtins are the most celebrated and studied active principles of neem oil due to their deterrent, antiovipositional, antifeedant, growth-disrupting, growth-regulating, fecundity, and fitness-reducing properties against insects and various kinds of arthropods (Ambrosino et al., 1999; Morgan, 2009). They are a group of closely related isomers that belong to the steroid-like tetranortriterpenoid class, called azadirachtin A to H (Rembold et al., 1984, 1987). Among all azadirachtins identified so far, azadirachtin A (azadirachtin) is a highly appreciated and interesting compound, as it is considered the most potent and principal agent for controlling insects (Sinha et al., 1999). Hence, it acts as a biomarker for standardization of neem oil and commercial insecticidal formulations (Sundaram and Curry, 1993; Sidhu et al., 2003). In 1968, Butterworth and Morgan isolated azadirachtin ($C_{35}H_{44}O_{16}$, MW 720, m.p., 160°C), and its synthesis was published 22 years after the discovery, due to its complicated structure (Gossé et al., 2005). The first total synthesis was given by Stevenley in 2007 (Veitch et al., 2007). The azadirachtin content in crude neem oil varies (100–4000 ppm), depending on the extraction technique, seed quality, environment, and genetic factors (Ambrosino et al., 1999; Ismadji et al., 2012). Neem seed kernel from Bali, Indonesia, is reported to contain up to 6200 ppm of azadirachtin.

### 20.4.4 Sterols

The major sterols in NSO reported were β-sitosterol, stigmasterol, campesterol, and fucosterol (Momchilova et al., 2007). The total tocopherols (298 ppm) indicate α-tocopherol and γ-tocopherol as major components (30.8% and 62.3%, respectively) (Djenontin et al., 2012).

## 20.5 Physical and Chemical Properties of Oil

Neem oil is normally a golden-yellow, yellowish-brown, reddish-brown, dark brown, greenish-brown, or bright red liquid. It has an unpleasantly strong and offensive odor. The smell of the oil is a partial combination of peanut and garlic. The obnoxious odor of neem oil is ascribed to the presence of sulfur-containing volatile compounds (Dasa Rao and Seshadri, 1941). It has an acrid taste, which is attributed to several triterpenoids present in it. It is a nondrying oil and, due to its hydrophobic nature, needs appropriate surfactants for proper emulsification during industrial application (Mongkholkhajornsilp et al., 2005; Usman et al., 2013; Edres, 2014). The quality of the oil depends on its composition, which in turn affects the properties. Since neem oil mainly contains fatty acids as one of the active

components, it is commonly analyzed for its quality by determining the saponification (SV), acid (AV), and iodine (IV) values, and so forth. Table 20.2 represents standard physicochemical properties of NSO (Okonkwo et al., 2013; Djibril et al., 2015).

The temperature has a considerable effect on the quality of NSO, as it decreases with an increase in temperature (Satyanandam et al., 2011). The poor quality of NSO can be confirmed by increased acid, saponification, and peroxide values (PVs) and decreased iodine value with high temperature (Liauw et al., 2008; Satyanandam et al., 2011). A high acid value indicates the presence of a high amount of free fatty acids in oil due to the degradation caused by hydrolysis at high temperature. The lipase enzymes in oil are responsible for the hydrolysis of triglycerides into free fatty acid and glycerol. Since the optimum temperatures for enzymes is 30°C–40°C, the extraction and storage temperatures are the chief concern in maintaining the quality of NSO (Khraisha, 2000; Choe and Min, 2006). The polarity of the extraction solvent also influences the AV, as low polarity caused the solvent to efficiently extract free fatty acids, thus escalating its acid value. According to Erakhrumen (2011), bio-oil with a high acid value can be exploited as a preservative for lignocellulose to enhance wood durability and properties.

The saponification value signifies the average molecular weight of the oil triglycerides. As high temperature causes lipid degradation, it reduces the average molecular weight of the NSO. The reduction in average molecular weight leads to a reduction in the viscosity of the oil. Also, the specific gravity (SG) or density of NSO decreases with an increasing temperature of exposure, and results in reduced viscosity and increased flow of oil. The peroxide value of oil implies rancidity and increases with temperature (Adeeko and Ajibola, 1990). Rancidity can be caused by hydrolysis, oxidation, or microbes. It gives an unpleasent smell to the oil due to the degradation of glycerides or formation of aldehydes and ketones. Oil with a high AV has poor resistance to peroxidation, particularly during storage. Increased extraction and storage temperature or long improper storage may lead to rancidity of NSO, thereby reducing its oxidative stability (Mongkholkhajornsilp et al., 2005). The iodine value expresses the degree of unsaturation in oil. The high temperature degrades the bonding, thereby leading to a decreased iodine value. Moreover, IV represents the drying nature of oil, where iodine values greater than 140 g/100 g and IVs less than 125 g/100 g are characteristics of drying and nondrying oil, respectively (Wicks et al., 1992). Unlike temperature, the polarity of the extraction solvent did not have any influence on SV and IV (Liauw et al., 2008; Erakhrumen, 2011). The results of some recent research on the physical and chemical properties of NSO are presented in Table 20.3.

**TABLE 20.2**

Standard Physicochemical Properties of Neem Seed Oil

| Properties | Value |
|---|---|
| Odor | Garlic |
| Specific gravity at 30°C | 0.908–0.934 |
| Viscosity at 37.8°C ($mm^2/s$) | 49.79 |
| Refractive index at 30°C | 1.4615–1.4705 |
| pH | 5.7–6.5 |
| Iodine value | 65–80 g/g |
| Acid value | 40 mg of KOH/g |
| Saponification value | 175–205 mg of KOH/g |

**TABLE 20.3**

Properties of NSO Obtained by Various Methods

| Extraction Method | AV (mg/g) | SV (mg of KOH/g) | IV (g/100 g) | PV (mg/g) | Reference |
|---|---|---|---|---|---|
| Cold pressing | 18.24 | 172.88 | 93.11 | 1.42 | Erakhrumen, 2011 |
| Solvent (hexane–ethanol, 60:40) | 12.90 | 199.99 | | | Ayoola et al., 2014 |
| Solvent (Soxhlet extractor, cyclohexane) | 10.2 | 200 | 72.82 | | Djibril et al., 2015 |
| Solvent (Soxhlet extractor, hexane) | 17.40 | 186.4 | 58.20 | 78.40 mEq/g | Zaku et al., 2012 |
| Solvent (hexane) | 1.411 g/g | 176.64 mg/g | 89.35 g/g | | Sodeinde and Samuel, 2014 |

## 20.6 General Uses of Oil

### 20.6.1 Medicine

The therapeutic application of neem had been identified by Indians 4000 years ago, since the Vedic period. In Sanskrit, the neem tree is recognized as "Arishtha," meaning "reliever of sickness." It is regarded as the "village dispensary" (Brahmachari, 2004). Traditionally, all parts of this divine tree have been utilized against various human ailments. According to Ayurvedic medicine, NSO has been used for the treatment and control of leprosy, syphilis, eczema, chronic ulcer, and intestinal helminthiasis (Hashmat et al., 2012). In the Siddha system of medicine, a preparation containing NSO, called *onan cutar tailam*, is used for epilepsy. However, apart from being practiced in traditional (Ayurvedic, Siddha, and Unani) medicine, there are several modern scientific investigations that have been carried out on NSO that have justified its therapeutic usage. NSO and its active components have displayed several pharmacological activities, including anti-inflammatory, antiarthritic, antipyretic, hypoglycemic, diuretic, spermicidal, antifungal, antibacterial, antigastric ulcer, antiviral, and antipsoriasis activities (Brahmachari, 2004).

### 20.6.2 Agriculture and Public Health

Besides curative abilities, neem oil has been reputed as a source of naturally occurring pesticide and insecticide (Ahmad et al., 2015; Jhalegar et al., 2015; Rodrigues et al., 2015; Sridharan et al., 2015). Utilization of NSO products as agrochemicals is remarkable. Pusa neem golden urea (PNGU), a urea–neem oil adduct, is one example of a neem-based agrochemical applied as a nitrification inhibiting agent. Moreover, NSO is also used for public health, mainly as an antilouse and antimalarial agent (Al-Quraishy et al., 2015). Neem oil mixed with coconut oil acts as an effective mosquito repellent (Brahmachari, 2004).

### 20.6.3 Personal Care Products

NSO and its components are utilized in toiletries and cosmetics such as skin care (soap, eczema cream, antiseptic cream, nail care, and balm), hair care (shampoo and hair oils), oral hygiene (toothpaste), and other household products (insect repellent spray and lotion, candles, wax,

and lubricants) (Hashmat et al., 2012; Shetty et al., 2016). Purified NSO is also exploited in nail polish and other cosmetics preparation (Shetty et al., 2016).

## 20.7 Pesticidal Uses of Oil

Neem oil is recognized as a powerful biopesticide and may offer a solution to global agricultural, environmental, and public health problems. The NSO allelochemicals are reported to have feeding and oviposition deterrence, repellency, growth disruption, reduced fitness, and sterility activities, and hence have been widely used in agricultural pest control (Brahmachari, 2004). In NSO, high concentrations of bioinsecticide limonoids are reported, mainly azadirachtin A, azadirachtin B, nimbin, and salannin (Stark and Walter, 1995). The most potent limonoid in NSO, azadirachtin, primarily acts as an insect repellent and insect growth regulator (IGR). Its structure is similar to that of insect hormones, "ecdysones," responsible for metamorphosis in insects. It is active at minute concentrations (1–10 ppm) and responsible for hindering the action of ecdysones, thus preventing the larvae from shedding their exoskeletons. Thus, azadirachtin alters their life cycle and inhibits the development of immature insects (Lokanadhan et al., 2012; Radwan and El-Shiekh, 2012). Also, NSO exhibits antifeedent and oviposition deterrent activity (Benelli et al., 2015). Antifeedent activity, credited to azadirachtin, nimbin, salannin, epoxyazadiradione, and melandriol, causes antiperistalitic movement in the alimentary canal and initiates a vomiting sensation in the insect (Esparza-Díaz et al., 2011). The nauseated feeling and inability to swallow do not allow insects to feed on NSO-treated surfaces. It checks feeding in approximately 200 types of insects at concentrations of 10–100 ppm. Similarly, NSO sprayed during storage does not allow female insects to lay eggs (Lokanadhan et al., 2012).

The broad-spectrum activity coupled with non-toxicity to mammals brands NSO as a perfect candidate for biopesticide treatment. As it is effective without being unacceptably hazardous to users and the environment, positive steps have been taken by the government to promote the use of neem biopesticide in agriculture and public health programs (Kaushik, 2004). The use of biopesticides is assumed to be a significant component of integrated pest management (IPM) for the realization of sustainable agriculture, due to their economic viability and eco-friendly nature. Hence, a tremendous amount of research has been conducted in the last few decades to exploit neem's pesticidal potential in agriculture and the public health sector (Table 20.4).

## 20.8 Advantages as a Pesticide

Neem essential oil plays an important role in pest management, and interest in neem pesticides has grown during the last few decades, as numerous pesticides have been restricted due to environment and food safety issues (Stark and Walter, 1995). The widespread understanding of the undesirable effects of synthesized pesticides on plants, soil, and nontargeted creatures has shifted interest toward readily available sources of biopesticides, that is, botanical pesticides. They are safe, degradable, and cheap (Brahmachari, 2004; Lokanadhan et al., 2012). The growing acceptence of neem essential oil–based pesticides has generated a great

**TABLE 20.4**

Recent Research on Neem Essential Oil for Agricultural and Public Health Pest Control

| Effected Organism | Pathogen/Disease | Treatment | Effects | Reference |
|---|---|---|---|---|
| *Mangifera indica* L. (Taimour mango) | Powdery mildew and malformation | Neem oil (1%) | Powdery mildew disease severity index (DSI) was 18.17%, more efficient against mango malformation | Ismail, 2016 |
| Humans | *Aedes aegypti* (dengue mosquito) | *Metarhizium anisopliae* (entomopathogenic fungi) + neem oil (0.001%) | 12% survival | Gomes et al., 2015 |
| Cowpea (Brazil) | *Spodoptera eridania* (southern armyworm) | Neem oil (0.35% and 0.7%) | Reduced leaf consumption | Rodrigues et al., 2015 |
| Brinjal | *Leucinodes orbonalis guenee* (shoot and fruit borer) | Neemarin (neem oil) (3 L/ha) | Reduced shoot and fruit damage | Singh and Sachan, 2015 |
| Humans | *Pediculus humanus capitis* (head louse) | Antilouse shampoo Licener® (shampoo + neem oil) | Oxygen uptake is prohibited in 3–10 min | Al-Quraishy et al., 2015 |
| Cowpea | *Maruca vitrata* | Multinucleopolyhedrovirus (MaviMNPV) + neem oil | Induced MaviMNPV infection in *M. vitrata* populations | Sokame et al., 2015 |
| Humans | *Anopheles arabiensis* (Ethiopian malaria mosquito) | Neem oil (20%) | More than 70% repellency (protection) in 3 h | Abiy et al., 2015 |
| Kinnow mandarin (*Citrus nobilis* × *Citrus deliciosa*) | *Penicillium digitatum* and *Penicillium italicum* | Neem essential oil | Influenced overall acceptability of postharvest crop | Jhalegar et al., 2015 |
| Cultivated crops | *Helicoverpa armigera* (moth) | Neem oil (1% emulsifiable concentrate [EC] azadirachtin) | Reduction in fecundity, reproductive rates, immature development | Ahmad et al., 2015 |
| Humans | *Aedes albopictus* (Asian tiger mosquito), filariasis | Neem seed oil | Larvicidal toxicity and field oviposition deterrence | Benelli et al., 2015 |
| Cotton | Cotton pest | *Beauveria bassiana* (entomopathogenic fungi) + neem oil | Damaged reproductive organs of pests | Togbé et al., 2015 |
| Kale plant | *Brevicoryne brassicae* (cabbage aphid) | Neem oil (1%) | Less cabbage aphid population | Pissinati and Ventura, 2015 |
| Okra (*Abelmoschus esculentus* L. Moench) | *Bemisia tabaci* (whitefly) | Mineral oil + neem oil (2%) | 95% mortality (after 48 h) | Sridharan et al., 2015 |

(*Continued*)

**TABLE 20.4 (CONTINUED)**

Recent Research on Neem Essential Oil for Agricultural and Public Health Pest Control

| Effected Organism | Pathogen/Disease | Treatment | Effects | Reference |
|---|---|---|---|---|
| Western white pine | *Zootermopsis augusticollis* (dampwood termite) | Neem oil | Rapid mortality | Fatima and Morrell, 2015 |
| Cashew trees | *Toxoptera odinae* (cashew aphid) | Neem oil | Killed 72.7%–78.9% of aphid population | Ambethgar, 2015b |
| Stone fruits | *Monilinia fructicola* | Neem oil (3.53 g/L) | 50% inhibition of mycelial growth | Lalancette and McFarland, 2015 |
| Watermelon | *Aphis gossypii* (watermelon aphid) | Neem oil | Dose-dependent decrease in population growth rate | Souza et al., 2015 |
| Humans | *Cimex lectularius* L. (bed bug) | Commercial neem oil | Killed 100% bed bugs | Feldlaufer and Ulrich, 2015 |
| Humans | *Aedes aegypti* (dengue mosquito) | Leaf essential oil | Effective against first-instar larvae and pupal stage (48 h) | Nasir et al., 2015a |
| Humans (contaminated food) | *Penicillium verrucosum* and *Penicillium nordicum* (produce ochratocin) | Neem essential oil (15 µl/ml) | 100% and 77.52%–92.49% inhibition, respectively | Koteswara Rao et al., 2015 |
| Coconut | *Aceria guerreronis* (coconut mite) | Neem oil (3%) | 31.31% reduction in mite population | Balaji and Hariprasad, 2015 |
| Cultivated crops | *Helicoverpa armigera* (moth/ cotton bollworm) | PONNEEM (neem + pongam oils, 1:1 ratio) (20 ppm) | Feeding deterrence and genotoxicity | Packiam et al., 2015 |
| Humans | *Aedes aegypti* and *Aedes albopictus* | Neem essential oil (10% solution with canola oil) | Insect repellency of 246 ± 15.78 and 256 ± 14.87 min | Nasir et al., 2015b |
| *Jasminum auriculatum* | *Aceria jasmini* (eriophyid mite) | Neem oil (30 ml/L) | Reduced mite population | Devi et al., 2015 |
| Tomato | Whitefly and leaf miner | Neem oil (2.5 L/ha) | Effective after 20 days of transplanting | Chavan et al., 2015 |
| Cashew | *Ferrisia virgata* (Cockerell) | Neem oil | Effective to a limited extent after two rounds of spraying at 7-day intervals | Ambethgar, 2015a |
| Okra | *Bemisia tabaci* Genn. (whitefly), yellow vein mosaic | Neem oil | 8.89% of disease intensity | Kumar et al., 2015 |
| Tomato | *Tuta absoluta* Meyrick (tomato moth) | Neem seed oil | Significant effect recorded | Salem and Abdel-Moniem, 2015 |
| *Phaseolus vulgaris* L. (dry bean plants) | *Bemisia tabaci* Genn. biotype B | Neem oil | High nymphal mortality (>81%) was achieved | de Almeida Marques et al., 2015 |

prospect for producers to exploit the same for commercial gain, and plentiful researches have been directed toward the safety and efficacy evaluation of neem pesticides (Boeke et al., 2004; Anis Joseph et al., 2010; Vethanayagam and Rajendran, 2010). NSO pesticide has exhibited very low toxicity toward most vertebrates, and no significant adverse effect on the ecosystem has been observed (Sinha et al., 1999; Ismadji et al., 2012). A clinical study conducted on adults (156) and children (110) did not show any side effects on them after 1 year of exposure to neem oil (1%) (Brahmachari, 2004). Among all the neem-based products, NSO is considered an extremely safe insecticide to protect stored seeds for human consumption (Boeke et al., 2004). Thus, NSO is generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) for use in food products. Also, they exempted NSO from the requirement of a maximum allowable pesticide limit on agricultural products. Neem pesticides are eco-friendly and environmentally friendly because their components rapidly biodegrade in the presence of sunlight and do not leave any residue on terrestrial and aquatic environment (Radwan and El-Shiekh, 2012). The half-life of azadirachtin (the main active component of NSO) on plants, soil, and water has been reported as 1–2.5 days, 3–44 days, and 48 min to 4 days, respectively. The remaining components of NSO are disintegrated by microbes present in the soil and water bodies. Additionally, the treated pests do not develop any resistance for NSO after prolonged use, as it modifies their life cycle instead of killing them. A remarkable feature of neem oil pesticides is their selective approach toward pests, as they do not harm beneficial insects, predators, parasites, and species that assist in pollination. Instead, they target only the chewing and sucking-type insects feeding on plants and animals, respectively (Radwan and El-Shiekh, 2012). Moreover, NSO can nourish and condition the soil and should be used, along with other pesticides and oils, for more efficacy (Lokanadhan et al., 2012).

## 20.9 Limitations as a Pesticide

Although NSO is a GRAS biopesticide, side effects in humans, animals, fish, and some nontargeted insect species have been reported in a number of isolated cases (Table 20.5) (El-Badawi et al., 2015). NSO is slightly irritating to the eyes, skin, and stomach due to the presence of azadirachtin (Shetty et al., 2016). In some instances, NSO in humans showed acute toxicity after oral administration (Boeke et al., 2004). Lai et al. (1990) reported that an even small amount of NSO can cause toxic encephalopathy, where vomiting, drowsiness, tachypnea, recurrent generalized seizures, leukocytosis, and metabolic acidosis are the main symptoms. In one extreme report, a child expired after oral administration of NSO for cough treatment. Autopsy findings revealed alterations in the liver and kidney of the child, consistent with Reye's syndrome (Sinniah et al., 1982). These incidences imply that the dosage taken as a drug was very high for human consumption, and the safe dose should be below ±0.20 ml/kg of body weight (bw), as calculated from animal studies. Recently, Shetty et al. (2016) reported a case of an adult female who committed suicide by consuming NSO. In rats and rabbits, NSO causes damage to the central nervous system (CNS) and lungs at a lethal dose ($LD_{50}$) of 14.1 and 24.0 ml/kg of bw, respectively (Gangopadhyay, 1994). Many studies indicated an antifertility effect of NSO in humans, attributed to the salanin compound (Brahmachari, 2004). *In vitro* studies on neem essential oil exhibited spermicidal activity in rats (0.25 mg/ml), rhesus monkeys, and humans (25 mg/ml) (Riar et al., 1990). In females, NSO administration in rats

**TABLE 20.5**

Adverse Effect of NSO on Humans and Animals

| Effected Organism | Exposure | Dose | Observed Effects | Reference |
|---|---|---|---|---|
| Humans (old female) | Ingestion | | Multiple organ failure, toxic encephalopathy | Shetty et al., 2016 |
| *Ceraeochrysa claveri* (predator insect) | Intake of neem oil-contaminated prey, *Diatraea saccharalis* eggs | 0.5%, 1%, and 2% neem oil | Sublethal effect: Cytotoxic effects in the adult midgut | Scudeler et al., 2016 |
| *Oreochromis niloticus* (Nile tilapia fish) | Treated with NSO | 112.5 ppm | Interfered with the antioxidant defense system; decrease in GST, CAT, and SOD | El-Badawi et al., 2015 |
| Rats | Ingestion | 2.0, 3.3, 4.6 ml/kg of bw | Subacute toxicity: Antifertility in females | Dhaliwal et al., 1998 |
| Mice | Ingestion | 1.0–28.2 g/kg of bw | Acute toxicity | Tandan et al., 1995 |
| Humans (child) | Oral droplets | 5 ml | Toxic encephalopathy | Lai et al., 1990 |
| Rabbits, rats | Ingestion | 10–80 ml/kg of bw | Acute toxicity | Gandhi et al., 1988 |
| Humans (child) | Ingestion | 12 ml | Changes in the liver and kidneys | Sinniah et al., 1982 |

*Note:* CAT, catalase; GST, glutathione-S-transferase; SOD, superoxide dismutase.

suggested high abortive effects during early pregnancy (Lal et al., 1987). Since NSO is used to treat stored seeds against insects, much controversy exists on the claims of the negative effect on treated seeds. According to Naik and Dumbre (1985), NSO, being bitter in nature, affects the taste and influences the germination of treated seeds. On the contrary, no such adverse change in the taste of treated seeds was reported elsewhere (Boeke et al., 2004). Also, NSO can easily turn rancid and be contaminated by aflatoxins, which might pose additional health risks to consumers (Sinniah et al., 1982). Although neem oil poisoning is rare, it may provoke deleterious changes in vital organs of the organism, if exposed directly or indirectly. Hence, precautions must be taken while it is administered as a pest management tool.

## 20.10 Essential Oil–Based Insecticides

Neem has undergone extensive research as a bioinsecticide from the past three decades, specifically in the United States and other European countries. Ample scientific evidence has encouraged the formulation of several commercial products based on either NSO or its most active component, azadirachtin. The first marketable neem insecticide, Margosan O, was produced in the United States (W.R. Grace and Co., Columbia, Maryland). It is composed of 0.25% azadiractin and 3%–5% neem oil and has received exemption from the U.S. EPA (Radwan and El-Shiekh, 2012). Neemguard (W.R. Grace and Co.) is another commercial insecticide, consisting of formulated neem oil from neem seed kernel (Stark and Walter, 1995). Later, several neem formulations were prepared from neem oil and have found wide usage as a bioinsecticide for organic cultivation. Neem oil pesticide resists a wide variety

of pests including mealy bugs, beet armyworms, aphids, cabbage worms, thrips, whiteflies, mites, fungus gnats, beetles, moth larvae, mushroom flies, leaf miners, caterpillars, locusts, nematodes and the Japanese beetles (Table 20.4) (Ahmad et al., 2015; Jhalegar et al., 2015; Rodrigues et al., 2015; Sridharan et al., 2015). In India, neem oil–based insecticide is commercially manufactured, and applied in cotton, vegetables, fruit trees, coffee, tea, rice farming, and so forth (Ambrosino et al., 1999). The other well-known commercial neem-based formulations with azadirachtin as an active ingredient are Neemix (W.R. Grace and Co.), Nimbecidine, Neemgold, Econeem Plus, Econeem, Soluneem, Limonool, FortuneAza, and NeemAzal-F.

## 20.11 Conclusions

Neem essential oil–based pesticide is environmentally benign. It is selectively toxic, does not bioaccumulate, and has short persistence in the ecosystem, hence making it an ideal candidate for an integrated pest management program. Various scientific studies have shown that severe side effects were only encountered when NSO was consumed directly in large amounts. However, direct and indirect contact with children and lactating and pregnant women should be avoided. Although there is a meager possibility of neem essential oil, applied as pesticide, entering the food chain of humans, most NSO degrades rapidly—before it reaches the consumer. Since a few studies have indicated the possibility of adverse effects, care should be taken in the administration of NSO as a pest-controlling agent. Furthermore, NSO is easily contaminated with aflatoxin; thus, the aflatoxin concentration in NSO-based pesticides should be properly controlled to avoid an unnecessary risk factor. As far as neem essential oil extraction is concerned, the solvent extraction method is the preferred choice due to its low operational and economic cost. Among modern methods of extraction, SFE is of great interest for being a green, efficient, and effective method for the recovery of essential oils and active metabolites.

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