

## NEEM BASED PESTICIDE FORMULATIONS AND AGRO-ENTREPRENEURSHIP

Ashwini M. Charpe<sup>1</sup> and Mohini M. Dange<sup>2</sup><sup>1</sup> Assoc. Prof. (Pl.Path.), AICRP on PHET, Dr.PDKV, Akola (MS) – 444 104<sup>2</sup> Asstt. Prof. (Agril. Process Engg.), Dept. of APE, CAET, Dr.PDKV, Akola (MS) – 444 104

ashwinicharpe@yahoo.com

(MS Received: 24.11.2022; MS Revised:06.12.2022; MS Accepted: 07.12.2022)



MS 2977

(RESEARCH PAPER IN AGRICULTURE.)

**Abstract:**

*Neem (Azadirachta indica) is well studied tree specie that has repeatedly demonstrated immense pesticidal efficacy due to active ingredients like azadirachtin, nimbin, nimbicidin, etc. Neem based pesticide formulations are reported to control plant parasitic fungi, bacteria, virus, nematodes, insects and mites. Indian mainland provide suitable climate for the growth of A. indica. According to the Report of an Ad Hoc Panel of the Board on Science and Technology for International Development National Research Council (1992), neem has been planted in many parts of Asia, Africa and some other areas of the world. However over 60% of the entire neem population is in India (Neem Foundation, 1997). It may be a lucrative business to further grow neem tree through community plantings and social forestry and promote agro-entrepreneurships to develop pesticide formulations on commercial levels. In India, bio-pesticides represent only 4.2 per cent of the overall pesticide market which is expected to exhibit an impressive annual growth rate of around 10 per cent in the coming years. According to the estimates of Neem Foundation (a voluntary, independent and non-profit organization) stated by EXIM Bank report, there are about 20 million neem trees in India. As per industry estimates, neem bears 3.5 million tonnes of kernels every year, and from this, around 7 lakh tonnes of neem oil can be obtained. Therefore, there is immense potential of neem based products in India, which can be tapped if the medicinal plant, as part of agro-forestry and Integrated Rural Development Program (IRDP), is popularized and its value added products are rolled out through village industries, says the Bank report. Recently, few such initiatives are taken by farmers and they are coming up with organized neem farming.*

**Key words:** *Neem, Azadirachta indica, Bio-pesticide, Agro-entrepreneurship*

**Introduction**

Neem (*Azadirachta indica* L.) commonly known as Indian Neem Plant is an evergreen, hard tree native to Indian sub-continent. It can easily be grown in a nutrient poor soil and survive even in dry harsh conditions. Both leaves and fruit of neem plant are known to have bitter taste having fungicidal, insecticidal and nematocidal properties. A tetranorterpeneoid limonoid Azadirachtin and Saponins are responsible for the biopesticidal potential of this wonder plant. Azadirachtin not only has a unique mode of action against insects, it also has adverse effects on fungi, viruses, nematodes and protozoans (Raizada et al., 2001). Biopesticides are good alternative to the synthetic pesticides as they are eco-friendly and has low toxicity level to humans and non-target organisms (Copping and Menn 2000; Nathan et al., 2005). Neem is recognized as one of the most reliable botanical sources of biopesticides.

**Insecticidal Properties of Neem**

The principle component that has insecticidal activity in neem extracts is a limonoid, Azadirachtin. It has been evaluated as the most promising insecticide of botanical origin, used against more than 400 species of insects. Up till now nine types of azadirachtin has been identified in neem seed extract. Azadirachtin has a molecular formula C<sub>35</sub>H<sub>44</sub>O<sub>16</sub> and chemically it is a tetranorterpeneoid. Azadirachtin have many reactive functional groups which are closely associated with each other which makes it a highly oxidized limonoid (Ley, Denholm and Wood, 1993). The major component of neem seeds is azadirachtin A. Azadirachtin acts on the mitotic cells and blocks the microtubule polymerization in insects. Certain activities of genes and proteins are also altered by azadirachtin. Azadirachtin acts on endocrine and neuroendocrine systems which are important to regulate the developmental processes of insects. It may cause a reduction in feeding habit, suspend the molting process, larvae and pupae death and also cause sterility in the emerging adults, this all depend on the given dose and thereby lowers their population density. It has been revealed that the anti-proliferating effect of azadirachtin is due to blocking of cell cycle and induction of apoptosis (Huang et al., 2011). In addition to this nuclear DNA is directly damaged by azadirachtin and also binds to a large protein complex including heat shock protein 60 (Robertson et al., 2007). Among various agricultural pests neem is reported to suppress pine weevil in forestry,

rice leaf folder, *Plutella xylostella* on vegetables. Other target insect species include *Anopheles stephensi* (Lucantoni et al., 2006), *A. culicifacies* (Chandramohan et al., 2016), *Ceraeochrysa claveri* (Scudeler et al., 2013, 2014; Scudeler and dos Santos, 2013), *Cnaphalocrocis medinalis* (Senthil Nathan et al., 2006), *Diaphorina citri* (Weathersbee and McKenzie, 2005), *Helicoverpa armigera* (Ahmad et al., 2015), *Mamestra brassicae* (Seljasen and Meadow, 2006), *Nilaparvata lugens* (Senthil-Nathan et al., 2009), *Pieris brassicae* (Hasan and Shafiq Ansari, 2011), and *Spodoptera frugiperda* (Tavares et al., 2010). Arachnid targets include *Hyalomma anatolicum excavatum* (Abdel-Shafy and Zayed, 2002) and *Sarcoptes scabiei* var. *cuniculi* larvae (Xu et al., 2010). Nathan et al. [22] studied the effects of neem limonoids azadirachtin, salannin, deacetyl gedunin, gedunin, 17-hydroxyazadiradione and deacetyl nimbin on the enzymes present in the gut of the larvae of the rice leaf folder. The activities of acid phosphatases (ACP), alkaline phosphatases (ALP) and adenosinetriphosphatases (ATPase) of rice leaf folder larvae gut were affected when they were fed on rice leaves treated with limonoids. Azadirachtin proved to be most effective in inhibiting all enzymes. Subrahmanyam et al. (23) reported that the antifeedant azadirachtin is responsible for the reduction in food consumption in female *Locust migratoria*. Nathan [22] reported the effect of azadirachtin on the mid gut enzymatic profile of *Spodoptera litura* Fab. Similarly, the effect of neem limonoids on lactate dehydrogenase of an insect, rice leaf folder (*Cnaphalocrocis medinalis*) was also reported [Nathan et al., 2006]. *Tribolium castaneum* (Herbst) is one of the chief storage pests that rely on starch and is responsible for the severe loss of stored grains [Butterworth and Morgan, 1971]. As the insects are totally dependent on starch hydrolyzing enzymes for their survival, these enzymes are good candidates for the inhibition by plant derived molecules. Azadirachtin is an antifeedant, and is involved in the inhibition of glutathione synthesis and inhibition of various neuroendocrine processes in the brain of locust (*Schistocerca gregaria*) [Parangama, Connolly and Strang, 2003]. A computational dissection of *Tribolium castaneum* endoglucanase enzyme, deduced from *T. castaneum* genome, showed that there were five cysteines involved in the formation of disulphide bridges in the molecule. The disulfide bridges did not provide any protection to

endoglucanase active site and beta-1,4-endoglucanase activity of *T. castaneum* was inhibited by azadirachtin. In a study it was demonstrated that azadirachtin has a latent effect on *T. confusum* progenies as manifested by the reduced growth and development of immature and mature stages, and lesser production of adults [Najat, 2011]. Azadirachtin can also interfere in mitosis, in the same way as colchicine, and has direct histopathological effects on insect gut epithelial cells, muscles, and fatty tissues, resulting in restricted movement and decreased flight activity (Wilps et al., 1992; Mordue (Luntz) and Blackwell, 1993; Qiao et al., 2014). Moreover, azadirachtin can inhibit the release of prothoracicotropic hormone and allatotropins from the brain-corpus cardiacum complex, resulting in problems of fertility and fecundity (Mulla and Su, 1999).

Saponins also have insecticidal properties. Saponins were able to inhibit beta-1,4 endoglucanase enzyme activity, present in pine weevil in forestry, rice leaf folder and *P. xylostella* on vegetables. Repellancy test for *T. castaneum* revealed that each group of compounds (Saponins and Azadirachtin) was able to repel the insect. Saponins may be considered as a part of the plant defense system (Morrissey and Osbourn, 1993). It has been reported that saponins can be activated by the plant enzymes in response to tissue damage by insects (Gus-Mayer et al., 1994). Azadirachtin, salanin and other limonoids present in neem oil also inhibit ecdysone 20-monooxygenase, the enzyme responsible for catalyzing the final step in conversion of ecdysone to the active hormone, 20-hydroxyecdysone, which controls the insect metamorphosis process (Morgan, 2009). Meliantriol and salannin also act to inhibit the feeding of insects (EMBRAPA, 2008).

Several studies have described the action of neem oil in specific groups of insects. Among the major insect groups, neem oil has shown action against (i) Lepidoptera: anti-feeding effect and increased larvae mortality (Mancebo et al., 2002; Michereff-Filho et al., 2008; Tavares et al., 2010); (ii) Hemiptera: early death of nymphs due to inhibition of development and ecdysis defects (Weathersbee and McKenzie, 2005; Senthil Nathan et al., 2006; Formentini et al., 2016); (iii) Hymenoptera: food intake decrease, reduced larval and pupal development, larvae death during the molting process (Li et al., 2003); (iv) Neuroptera: severe damage in the midgut cells of larvae, injury and cell death during the replacement of midgut epithelium, and changes in cocoons, with increased porosity and decreased wall thickness affecting pupation (Scudeler et al., 2013, 2014; Scudeler and dos Santos, 2013). In another class, the Arachnida, exposure of the Ixodidae group to neem oil decreased egg hatching and caused malformation, deformities, and death of larvae and adults (Abdel-Shafy and Zayed, 2002).

#### Antifungal Properties of Neem

Fungi have also been reported sensitive to azadirachtin and saponins (Mordue (Luntz), Morgan and Nisbet, 2005). Saponins may be considered as a part of the plant defense system (Morrissey and Osbourn, 1993). It has been reported that saponins can be activated by the plant enzymes in response to pathogen attack (Gus-Mayer et al., 1994). Saponins are the surface-active glycosides that naturally occur in certain plants, animals and microorganisms. Mainly they are produced by plants but lower amount in marine animals and some bacteria. Saponins are named because of their soap like characteristics. Usually saponins consist of a sugar moiety containing glucose, galactose, glucuronic acid, xylose, rhamnose or methyl pentose (Wang et al., 2000). A glycosidic linkage is present between sugar moiety and hydrophobic aglycone that may be a terpenoid or steroid in nature. The aglycone could contain one or more C-C unsaturated bonds. Adepoju et al. (2014) studied the effects of Neem seed oil on four fungi, *Fusarium* sp. *Rhizopus* sp. *Curvularia* sp. and *Aspergillus* sp. which are pathogenic in nature. The crude extract of neem seed prepared using

petroleum ether was used that demonstrated suppression of *Fusarium* sp., *Curvularia* sp. and *Aspergillus* sp. Mulla and Su (1999) and Biswas et al. (2002) has also confirmed the antifungal activities of Neem oil. Da-Costa et al. (2010) confirmed the inhibition of fungal growth (i.e. mycelia dry weight, diameter of colony and growth rate) of *Aspergillus flavus* on solid media when Neem extract was used at concentrations of 0.5 to 5.0% v/v, although it significantly increased sporulation in the same conditions. Locke (1995), Martinez (2002) and Da-Costa et al. (2010) reported that due to the antifungal efficacy of neem seed extract, its biodegradability and minimum side effects, azadirachtin, a tetranortriterpenoid obtained from the seed has emerged as a natural biopesticide. They further confirmed that neem seed extracts had higher inhibition percentage than that of neem leaf extracts. There have been reports of the use of neem extract for phytopathogen control. For example, neem extract could inhibit the growth of *Alternaria solani* (which causes early blight disease in potatoes and tomatoes), *Fusarium oxysporum* (which causes Fusarium wilt disease in a variety of plants), *Rhizoctonia solani* (which causes damping-off in a variety of seedlings), and *Sclerotinia sclerotiorum* (which causes Sclerotinia, or white mold disease, in most vegetables) (Moslem and El-Kholie, 2009; Hassanien et al., 2010; Al-Hazmi, 2013). Al-Hazmi (2013) found that alcohol extracts of neem seeds could efficiently inhibit phytopathogens such as *Pythium aphanidermatum*, *Alternaria alternata*, and *Helminthosporium* sp. However, the neem seed extract created using a water extraction process was a poor inhibitor of the same phytopathogens.

#### Antihelminth Properties of Neem

Root-knot nematodes (*Meloidogyne* spp.) is the most important nematode pest of both tropical and sub-tropical regions. There are more than 2000 plant spp including herbaceous, wood plants of mono and dicotyledons which are being affected by nematodes that are obligate parasite of these plants. *M. incognita* and *M. javanica* cause most of the damage to field crops, vegetables and fruit trees. Neem leaves, oil cake and azadirachtin have been proved to have nematicide activity as they are reported to reduce number of eggs and egg mass of nematodes. Application of 0.1% w/w of azadirachtin is reported to reduce the invasion of juvenile nematodes (Javed et al., 2007). Sivakumar and Gunasekaran (2011) and Mohammad Akhtar (2000) has demonstrated the reduction of root knot nematode population by 48.6, 51.7 and 39.6 per cent over control on tomato, chilli and brinjal, respectively when neem oil formulation No 60 EC (C) was used as seed treatment and seedling root dip @ 2ml / lit. .

#### Antiviral Properties of Neem

Viruses have also been reported sensitive to azadirachtin (Mordue (Luntz), Morgan and Nisbet, 2005). Among various limonoids nimbin and nimbidin mainly present antiviral activity (Embrapa, 2008). Transmission of potato virus Y to sweet pepper by the green peach aphid, *Myzus persicae* (Sulzer), was inhibited by foliar applications of 1.0% or 2.0% neem seed oil to infected source plants or to uninfected recipient plants. Neem seed oil interfered with virus acquisition and inoculation in a manner comparable to that of commercial horticultural oil, while an oil-free neem seed extract did not reduce rates of transmission compared with controls. The finding that neem seed oil inhibits virus transmission, while oil-free neem seed extract does not, suggests that the presence of the oil rather than biologically active limonoids such as azadirachtin interfere with virus transmission. None of the treatments affected rates of infection when potato virus Y was transmitted mechanically, or the resulting virus titre and symptom expression. In addition to direct control of insect pests, formulated neem oils may help reduce or delay the spread of non-persistent plant viruses (Lowery, Eastwell and Smirle, 2008).

#### Other target organisms (Bacteria, Protozoa & Mites)

According to Mulla and Su (1999) and Biswas et al (2002), neem oil, extracted from the seeds of *A. Indica*, has versatile medicinal properties, including antibacterial activities against clinical bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*, and against phytopathogenic bacteria such as *Xanthomonas vesicatoria*, *Ralstonia solanacearum* (Sukanya et al., 2009; Al-Hazmi, 2013; Jain et al., 2013). Protozoa have also been reported sensitive to azadirachtin (Mordue (Luntz), Morgan and Nisbet, 2005). Azadirachtin have also been explored as inhibitor of sexual development of malaria parasite and larvicidal against mosquito larvae. It is also being investigated for having inhibitory effect on replication of dengue virus type-2. Chloroform extracts and petroleum ether extracts of neem oil have also been found to exhibit potent acaricidal activity against *Sarcoptes scabiei* var. cuniculi larvae (Du et al., 2008, 2009). According to Mulla and Su (1999) and Biswas et al.(2002), neem oil, extracted from the seeds of *A. indica*, has versatile medicinal properties, including antifertility, antifungal, antibacterial, immunostimulant, antipyretic and acaricidal activities.

#### Effect on Non-targeted organisms

As a biopesticide Azadirachtin is known to have very little effect on non-targeted organisms for example pollinators (Mordue (Luntz), Morgan and Nisbet, 2005; Mordue (Luntz), 1993; Naumann and Isman, 1996). It is completely nontoxic to vertebrates (Salehzadeh et al, 2002). Neem products have very low toxicity level against human beings (Nathan et al., 2009). Neem based insecticides are among the least toxic insecticides to humans and shows very low toxicity to beneficial organisms. Usually pests do not become resistant to this pesticide (Feng and Isman, 1995). Sahayaraj et al. (2011) evaluated the use of different neem-based products in colonies of *Beauveria bassiana*, *Isaria fumosoroseus*, and *Lecanicillium lecanii*, and the results showed that these entomo-pathogenic fungi were compatible with most products tested. Raguraman and Kannan (2014) conducted a review in order to score the impact and safety of different botanical insecticides in the presence of parasitoids and predators (beneficial arthropods), with the aim of standardizing strategies and application methods to achieve better management of agricultural pests. Neem extract and pure azadirachtin are both considered nontoxic to beneficial organisms such as earthworms, and safe for human consumption (Khalid and Shad, 2002; Boeke et al., 2004).

#### Effect of Neem on Beneficial Soil Microbes

Sarawaneeyaruk, Krajangsang and Pringsulaka (2015) found that both azadirachtin and neem extract usage reduce the number of root nodules on mung bean plants, as well as the *T. Asperellum* population in the rhizosphere. Azadirachtin in particular reduced the populations of soil and rhizosphere microorganisms. Therefore, the use of azadirachtin and neem extract should be supplemented with organic fertilizer or the application of effective microorganisms.

#### Biopesticide Preparation from Neem

Neem oil is extracted from the neem tree, *A. indica* Juss., a member of the *Meliaceae* family that originates from the Indian subcontinent and is now valued worldwide as an important source of phytochemicals for use in human health and pest control. Neem oil contains at least 100 biologically active compounds. Among them, the major constituents are triterpenes known as limonoids, the most important being azadirachtin (which appears to cause 90% of the effect on most pests. The compound has a melting point of 160°C and molecular weight of 720 g/mol. Other components present include meliantriol, nimbin, nimbidin, nimbinin, nimbolides, fatty acids (oleic, stearic, and palmitic), and salannin. The main neem product is the oil extracted from the seeds by different techniques. The other parts of the neem tree contain less azadirachtin, but are also used for oil extraction (Norten and Putz, 1999; Nicoletti et al., 2012; Forim et al., 2014). Products derived from neem can

contribute to sustainable development and the resolution of pest control problems in agriculture (Lokanadhan et al., 2012). Neem products can effectively contribute to organic agriculture, being used as organic pesticides and as soil fertilizers. In addition, growing concerns about conventional agriculture and the demand for products that do not generate waste justify increased adoption of the use of biopesticides by farmers, which contributes to the growth of organic agriculture (Dubey et al., 2010; Seufert et al., 2012; Gahukar, 2014).

#### Limitations of Neem Based Formulations

Despite its many promising properties, there are limitations that hinder effective large-scale use of neem. These impediments must be overcome and many uncertainties clarified so that the full potential of neem can be exploited. One of the main problems facing the commercial development of neem is a lack of industrial interest, largely due to the difficulty of patenting natural products, as well as a shortage of scientific evidence to support claims regarding the benefits of these substances. As a results, the products are not widely publicized in the farming community and elsewhere (Pant et al., 2016). Disadvantages of neem are its low stability under field conditions, due mainly to a high rate of photodegradation, as well as a short residence time and slow killing rates, compared to conventional pesticides (Isman, 2006; de Oliveira et al., 2014; Miresmailli and Isman, 2014). Genetic factors are mainly responsible for determining the chemical composition of neem oil. However, environmental factors and the type of extraction method can lead to significant differences in composition. As a result, there is no standard active ingredient in the composition of this botanical insecticide, which limits its application in the control of agricultural pests (Ghosh et al., 2012; Tangtrakulwanich and Reddy, 2014; Siegwart et al., 2015). Neem oil contains a group of active ingredients with different chemical characteristics. It was therefore believed that the development of insect resistance would be virtually impossible. However, as studies have progressed, it has been observed that due to the low residual power of botanical insecticides, multiple applications are required in order to control pests, which can increase selection pressure on the pest population, possibly leading to resistance (Ghosh et al., 2012; Tangtrakulwanich and Reddy, 2014; Siegwart et al., 2015). Currently, most of the botanical insecticides that are being studied and that are effective against many pests are those with feeding deterrent action, so their indiscriminate use could result in the development of resistance (Tangtrakulwanich and Reddy, 2014; Mpumi et al., 2016). Feng and Isman (1995) evaluated the behavior of two lines of *Myzus persicae*, which were exposed to pure azadirachtin or to refined neem seed extract at the same concentration as azadirachtin. It was found that after forty generations, the line treated with azadirachtin had developed ninefold greater resistance to azadirachtin, compared to a control line, whereas the line treated with the extract did not show resistance.

#### Nanoencapsulation to Overcome Limitations of Neem Based Formulations

In order to overcome the above-mentioned limitations, nanotechnology has emerged as a novel tool to address the problems of agricultural sustainability and food security (Khot et al., 2012; Kah and Hofmann, 2014; Kookana et al., 2014; Kah, 2015; Kashyap et al., 2015; Fraceto et al., 2016). Many studies have shown that the encapsulation of agrochemicals in nanoparticulate systems can enhance the efficacy of the active ingredient, decrease toxicity toward the environment and humans, and reduce losses due to volatilization, leaching, and photobleaching (Kulkarni et al., 1999; Riyajan and Sakdapipanich, 2009; Devi and Maji, 2010; de Oliveira et al., 2014; Bakry et al., 2016; Giongo et al., 2016). From the point of view of sustainable agriculture, nanotechnology can help in the development of environmentally friendly agricultural inputs, improving the safety and



stability of active agents, enhancing their activity in pest control, and, consequently, increasing their acceptance by producers (Nair et al., 2010; Srilatha, 2011; Khot et al., 2012; Agrawal and Rathore, 2014; Ram et al., 2014). The use of nanoparticles provides an effective means of protecting neem oil against premature degradation, resulting in prolongation of its effect on the target pest. Sustained release of the active agent is achieved, and environmental damage is minimal because the polymers employed are biodegradable. Furthermore, the number of applications of neem oil can be reduced, bringing substantial economic benefits (Kulkarni et al., 1999; Isman et al., 2001; Isman, 2006; de Oliveira et al., 2014; Isman and Grieneisen, 2014; Miresmailli and Isman, 2014). Although studies have demonstrated the beneficial effects of nanoencapsulation of neem oil, some issues need to be resolved so that the synergistic effect of nanoparticles associated with this botanical insecticide can significantly contribute to the control of insect pests. These issues include the need for: (a) regulation of the use of nanomaterials in agriculture; (b) nanoformulations that are easily scalable; (c) comparative studies employing neem formulations available commercially to prove the cost/benefit of nanoformulations; (d) detailed studies of the degradation and behavior of these nanopesticides in the environment; and (e) evaluation of toxicity toward non-target organisms (De Jong and Borm, 2008; Servin and White, 2016). Given the importance of neem oil and its worldwide use for combating numerous pests in different crops, the nanoencapsulation of this oil should enable the production of more stable formulations for the control of insects that damage crops, especially those that are essential for human consumption. In addition, the use of nanotechnology is an excellent way to combat the development of resistance in insects due to the indiscriminate use of neem oil.

#### Neem Based Agro-enterprizes

In many Asian countries neem is still being used as pest controlling agent for protecting plant in rural environment. In the western world the formulation of neem extract have been approved to be used in the management of pests in USA and many other European countries. Currently no neem products have been licensed for use in UK forestry or agriculture, but the register of organic food standard of UK has mentioned the neem extract as an acceptable product that could be used within organic farming operations. At present, within UK formulations of neem extract have been approved for use as repellent mixed with various lotions shampoos and soaps for human use. It has been revealed that neem extract play a major role in protecting seedling confers from attack of *H. abietis*. These neem formulations are environment friendly and it can reduce the use of synthetic pesticides (Thackera et al., 2003). Neem has acquired commercial recognition due to its various beneficial properties, which have been extensively investigated over time. Compared to conventional chemicals, which are generally persistent in the environment and highly toxic, botanical pesticides are biodegradable and leave no harmful residues. Most botanical pesticides are non-phytotoxic and are also more selective toward the target pest. In terms of commercial applications, biopesticides can provide substantial economic advantages, since the infrastructure required is inexpensive, compared to conventional pesticides (Pant et al., 2016). Several patents related to processes and products based on neem have been deposited in the United States, India, Japan, Australia, and elsewhere. Many of the products derived from neem are manufactured by crushing the seeds and other plant parts, followed by the use of solvents to extract the active ingredients possessing pesticide activity. The different methods and techniques employed to obtain neem products can result in different concentrations of the active compounds, as well as different biological effectiveness (Roychoudhury, 2016). The main application of neem oil is in agriculture. In the agriculture industry, neem oil is generally used

as a natural pesticide, insecticide, and fungicide. Containing different ppm azadirachtin in neem oil, it is used for a different purpose in different dosages. Another industrial use is neem coated chemical fertilizer like urea. Since neem is native to Indian sub-continent there is tremendous potential to establish agro-enterprizes to extract neem oil and azadirachtin for supply to agro-industries and to develop product based agro-enterprizes to develop biopesticide formulations for local as well as global markets. Other than agriculture neem oil has wide market potential in the cosmetic industry. In manufacturing skincare, haircare, body care product s, neem oil is an essential raw material. In the Ayurveda cosmetic industry which is now booming, neem oil is an essential ingredient. Neem oil is also an essential product in the herbal medicine industry.

The global neem extracts market demand was 503.5 USD million in 2013 and is projected to grow at a CAGR of 14.8% from 2014 to 2020. The market is expected to witness growth on account of rising demand from various end-use industries including personal care, pharmaceutical, animal feed, and agriculture. Furthermore, increasing consumer awareness about the health benefits of neem, particularly in developed regions, is anticipated to boost the market growth over the forecast period. The global neem extract market generated revenue worth \$653.7 million in 2015 and is expected to register a CAGR of 16.3% during the forecast period (2016–2022). USA and Italy are the leading importers of neem extracts from India. With USD 2.62 million imports in 2011-12, USA was the largest importer of neem extracts from India, according to the report. Japan was the largest importer of neem oil cakes from India, valued at USD 0.28 million in 2011-12, and Spain the largest importer of neem seeds from India. According to the estimates of Neem Foundation (a voluntary, independent and non-profit organisation), stated by the EXIM Bank report there are about 20 million neem trees in India,. As per industry estimates, neem bears 3.5 million tonnes of kernels every year, and from this, around 7 lakh tonnes of neem oil can be obtained. There is immense potential of neem based products in India, which can be tapped if the medicinal plant, as part of agro-forestry and Integrated Rural Development Program (IRDP), is popularised and its value added products are rolled out through village industries, says the Bank report. However, total neem oil produced in India is about 2.5 lakh tonnes, which is only 30 per cent of the total potential, the report said, indicating the scope for optimising yield. In India, bio-pesticides represent only 4.2 per cent of the overall pesticide market which is expected to exhibit an impressive annual growth rate of around 10 per cent in the coming years (Business news, 2013).

#### Conclusion

In the past few decades neem based pesticides are gaining more attention because of their non-toxic and environmental friendly nature. Azadirachtin and many other neem products have shown very impressive results as biopesticide against many diseases and pests of agricultural importance. There are few issues associated with the commercial use of neem based pesticides like photosensitivity of azadirachtin has to be managed for its long lasting effectiveness. Agro-industries based on neem oil and azadirachtin extraction as well as preparation of neem based pesticide and other formulations may create a boom in start-up ecosystem of India fetching global attention and demand.

#### References

1. Abdel-Shafy S and Zayed AA (2002). In vitro acaricidal effect of plant extract of neem seed oil (*Azadirachta indica*) on egg, immature, and adult stages of *Hyalomma anatolicum excavatum* (Ixodoidea: Ixodidae). *Vet. Parasitol.* 106: 89–96. Adepoju Adeyinka Olufemi, Ogunkunle Adepoju Tunde Joseph and Femi-Adepoju

- Abiola Grace (2014). Antifungal activities of seed oil of neem (*Azadirachta indica* A. Juss.) G.J.B.A.H.S., 3(1):106-109.
2. Agrawal S and Rathore P (2014). Nanotechnology pros and cons to agriculture: a review. *Int. J. Curr. Microbiol. Appl. Sci.* 3: 43–55.
3. Ahmad S, Ansari MS and Muslim M (2015). Toxic effects of neem based insecticides on the fitness of *Helicoverpa armigera* (Hübner). *Crop Prot.* 68: 72–78.
4. Al-Hazmi RHM (2013). Effect of neem (*Azadirachta indica*) leaves and seeds extract on the growth of six of the plant disease causing fungi. *Glo. Adv. Res. J. Microbiol.* 2: 89–98.
5. Bakry AM, Abbas S, Ali B, Majeed H, Abouelwafa MY, Mousa A, et al. (2016). Microencapsulation of oils: a comprehensive review of benefits, techniques, and applications: encapsulation of marine, vegetable, essential oils. *Compr. Rev. Food Sci. Food Saf.* 15: 143–182.
6. Biswas K, Chattopadhyay I, Banerjee RK and Bandyopadhyay U (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Sciences* 82 (11): 1336–1345.
7. Biu AA, Yusufu SD, Rabo JS (2009). Phytochemical screening of *Azadirachta indica* (Neem)(Meliaceae) in Maiduguri, Nigeria. *Biosci Res Comm.* 21: 281-283.
8. Boeke SJ, Boersma MG, Alink GM, van Loon JJ, van Huis A, Dicke M, Rietjens IM (2004). Safety evaluation of neem (*Azadirachta indica*) derived pesticides. *J Ethnopharmacol.* 94: 25-41.
9. Business News (2013). Neem-based pesticide market growing at 7-9% annually: Report, Ahmedabad: Sep 02, 2013.
10. Butterworth JH, Morgan ED (1971). Investigation of the locust feeding inhibition of the seeds of the neem tree, *Azadirachta indica*. *J Insect Physiol.* 17: 969-977.
11. Chandramohan B, Murugan K, Madhiyazhagan P, Kovendan K, Kumar PM, Panneerselvam C, et al. (2016). Neem by-products in the fight against mosquito-borne diseases: biotoxicity of neem cake fractions towards the rural malaria vector *Anopheles culicifacies* (Diptera: Culicidae). *Asian Pac. J. Trop. Biomed.* 6: 472–476.
12. Copping LG, Menn JJ (2000). Biopesticides: a review of their action, applications and efficacy. *Pest Manag Sci.* 56: 551-576.
13. Da-Costa Christiane L, Marcia RF Geraldo, Carla C. Arrotéia, Carlos Kemmelmeier (2010). In vitro activity of neem oil [*Azadirachta indica* A. Juss (Meliaceae)] on *Aspergillus flavus* growth, sporulation, viability of spores, morphology and Aflatoxins B1 and B2 production. *Advances in Bioscience and Biotechnology.* 1: 292-299.
14. De Jong WH and Borm PJ (2008). Drug delivery and nanoparticles: applications and hazards. *Int. J. Nanomed.* 3: 133–149.
15. de Oliveira JL, Campos EVR, Bakshi M, Abhilash PC and Fraceto LF (2014). Application of nanotechnology for the encapsulation of botanical insecticides for sustainable agriculture: prospects and promises. *Biotechnol. Adv.* 32: 1550–1561.
16. Devi N and Maji TK (2010). Genipin crosslinked microcapsules of gelatin A and  $\kappa$ -carrageenan polyelectrolyte complex for encapsulation of Neem (*Azadirachta Indica* A. Juss.) seed oil. *Polym. Bull.* 65: 347–362.
17. Du YH, Li JL, Yin ZQ, Li XT, Jia RY, Lv C, Zhang YQ and Zhang L (2009). Acaricidal activity of four fractions and octadecanoic acid-tetrahydrofuran-3,4-diyl ester isolated from chloroform extracts of neem (*Azadirachta indica*) oil against *Sarcoptes scabiei* var. cuniculi larvae in vitro. *Veterinary Parasitology.* 163 (1–2): 175.
18. Du YH, Jia RY, Yin ZQ, Pu ZH, Chen J, Yang F, Zhang YQ (2008). Acaricidal activity of extracts of neem (*Azadirachta indica*) oil against the larvae of the rabbit mite *Sarcoptes scabiei* var. cuniculi in vitro. *Veterinary Parasitology.* 157: 144–148.
19. Dubey RC, Kumar H and Pandey RR (2009). Fungitoxic effect of neem extracts on growth and sclerotial survival of *Macrophomina phaseolina* in vitro. *J. Am. Sci.* 5: 17–24.
20. EMBRAPA (2008). *A Cultura do Nim/Embrapa Florestas*, 1st Edn. Brasília: Embrapa Informação Tecnológica.
21. Feng R and Isman MB (1995). Selection for resistance to azadirachtin in the green peach aphid, *Myzus persicae*. *Experientia.* 51: 831–833.
22. Forim MR, Fernandes DSMF, Fernandes JB and Vieira PC (2014). *Processo de Obtenção de Nanopartículas Biopoliméricas Contendo Óleo e Extratos de Azadirachta Indica a. Juss (neem), Nanopartículas Biopoliméricas e Micropartículas em pó.*
23. Formentini MA, Alves LFA and Schapovaloff ME (2016). Insecticidal activity of neem oil against *Gyropsylla spegazziniana* (Hemiptera: Psyllidae) nymphs on Paraguay tea seedlings. *Braz. J. Biol.*
24. Fraceto LF, Grillo R, de Medeiros GA, Scognamiglio V, Rea G and Bartolucci C (2016). Nanotechnology in agriculture: which innovation potential does it have? *Front. Environ. Sci.* 4:20.
25. Francis G, Kerem Z, Makkar HPS and Becker K (2002). The biological action of saponins in animal systems: A review. *Br J Nutr.* 88: 587-605.
26. Gahukar RT (2014). Factors affecting content and bioefficacy of neem (*Azadirachta indica* A. Juss.) phytochemicals used in agricultural pest control: a review. *Crop Prot.* 62: 93–99.
27. Gangadhara Kumar PR and Prakash V (2009). Inhibition of rice bran lipase by azadirachtin from *Azadirachta indica*. *J Sci Food Agric.* 89: 1642-1647.
28. Ghosh A, Chowdhury N and Chandra G (2012). Plant extracts as potential mosquito larvicides. *Indian J Med Res.* 135: 581-598.
29. Giongo AMM, Vendramim JD and Forim MR (2016). Evaluation of neem-based nanoformulations as alternative to control fall armyworm. *Ciênc. Agrotec.* 40: 26–36.
30. Hasan F and Shafiq Ansari M (2011). Toxic effects of neem-based insecticides on *Pieris brassicae* (Linn.). *Crop Prot.* 30: 502–507.
31. Hassanien NM, Zeid MAA, Youssef KA and Mahmoud DA (2010). Control of tomato early blight and wilt using aqueous extract of neem leaves. *Phytopathol. Mediterr.* 49: 143–151.
32. Huang JF, Shui KJ, Li HY, Hu Y and Zhong GH (2011). Antiproliferative effect of azadirachtin-A on *Spodoptera litura* S1-1 cell line through cell cycle arrest and apoptosis induced by up-regulation of p53. *Pestic Biochem Physiol.* 99: 16-24.
33. Isman MB (2006). Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* 51: 45–66.
34. Isman MB and Grieneisen ML (2014). Botanical insecticide research: many publications, limited useful data. *Trends Plant Sci.* 19: 140–145.

33. **Jain D, Jayaram L, Prabhu MV and Bhat KG (2013).** Antibacterial effect of neem (*Azadirachta indica*) oil on multidrug resistant bacteria isolated from human infections. *Int. J. Biol. Med. Res.* 4: 3544–3546.
34. **Javed N, Gowen SR, Inam-ul-Haq M and Anwar SA (2007).** Protective and curative effect of neem (*Azadirachta indica*) formulations on the development of root-knot nematode *Meloidogyne javanica* in roots of tomato plants. *Crop Protection*. 26: 530-534.
35. **Kah M (2015).** Nanopesticides and nanofertilizers: emerging contaminants or opportunities for risk mitigation? *Front. Chem.* 3: 64.
36. **Kah M and Hofmann T (2014).** Nanopesticide research: current trends and future priorities. *Environ. Int.* 63: 224–235.
37. **Kashyap PL, Xiang X and Heiden P (2015).** Chitosan nanoparticle based delivery systems for sustainable agriculture. *Int. J. Biol. Macromol.* 77: 36–51.
38. **Khalid S and Shad RA (2002).** Potential advantage of recent allelochemical discoveries and agro-ecosystems. *Prog. Farm.* 11: 30–35.
39. **Khot LR, Sankaran S, Maja JM, Ehsani R and Schuster EW (2012).** Applications of nanomaterials in agricultural production and crop protection: a review. *Crop Prot.* 35: 64–70.
40. **Kookana RS, Boxall ABA, Reeves PT, Ashauer R, Beulke S, Chaudhry Q, et al. (2014).** Nanopesticides: guiding principles for regulatory evaluation of environmental risks. *J. Agric. Food Chem.* 62: 4227–4240.
41. **Kumar CSSR, Srinivas M and Yakkundi S (1996).** Limonoids from the seeds of *Azadirachta indica*. *Phytochem.* 43: 451-455.
42. **Lokanadhan S, Muthukrishnan P and Jeyaraman S (2012).** Neem products and their agricultural applications. *J. Biopestic.* 5: 72–76.
43. **Lowery D Thomas, Ken C Eastwell and M J Smirle (2008).** Neem seed oil inhibits aphid transmission of potato virus Y to pepper. *Annals of Applied Biology*, 130(2): 217-225.
44. **Lucantoni L, Giusti F, Cristofaro M, Pasqualini L, Esposito F, Lupetti P, et al. (2006).** Effects of a neem extract on blood feeding, oviposition and oocyte ultrastructure in *Anopheles stephensi* Liston (Diptera: Culicidae). *Tissue Cell.* 38: 361–371.
45. **Lynn OM, Kim JE and Lee KY (2012).** Effect of azadirachtin on development and gene expression of fifth instar larvae of Indianmeal moth, *Plodia interpunctella*. *J Asia-Pacific Entomology*. 15: 101-105.
46. **Martinez SS (2002).** O Nim-Azadirachta indica. Natureza, Usos Múltiplos, Produção. In: Martinez, S.S. Ed., IAPAR, Londrina, PR, Brazil.
47. **Michereff-Filho M, Torres JB, Andrade LN and Nunes MUC (2008).** Effect of some biorational insecticides on *Spodoptera eridania* in organic cabbage. *Pest Manag. Sci.* 64: 761–767. Miresmailli S and Isman MB (2014). Botanical insecticides inspired by plant–herbivore chemical interactions. *Trends Plant Sci.* 19: 29–35
48. **Mohammad Akhtar (2000).** *Integrated Pest Management Reviews*. 5: 57–66
49. **Mordue (Luntz) AJ, Morgan ED and Nisbet AJ (2005).** Azadirachtin, a natural product in insect control. In: Gilbert, L.I., Iatrou, K., Gill, S.S. (Eds.), *Comprehensive Molecular Science*. Elsevier, Oxford. 6: 117-135.
50. **Morgan ED (2009).** Azadirachtin, a scientific gold mine. *Bioorg. Med. Chem.* 17: 4096–4105.
51. **Moslem MA and El-Kholie EM (2009).** Effect of neem (*Azadirachta indica* A. Juss) seeds and leaves extract on some plant pathogenic fungi. *Pak. J. Biol. Sci.* 12: 1045–1048.
52. **Mpumi N, Mtei K, Machunda R and Ndakidemi PA (2016).** The toxicity, persistence and mode of actions of selected botanical pesticides in Africa against insect pests in common beans, *P. vulgaris*: a review. *Am. J. Plant Sci.* 7: 138–151.
53. **Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y and Kumar DS (2010).** Nanoparticulate material delivery to plants. *Plant Sci.* 179: 154–163.
54. **Najat Aly Khatteer (2011).** Efficiency of azadirachtin, a chitin synthesis inhibitor on growth, development and reproductive potential of *Tribolium confusum* after adult treatment. *J. Entomol.* 8: 440-449.
55. **Nathah SS, Choi MY, Paik CH, Seo HY and Kalaivani K (2009).** Toxicity and physiological effects of neem pesticide applied to rice on the *Nilaparvata lugens* sta 1, the brown plant hopper. *Ecotoxicol Environ Safety.* 72: 1707-1713.
56. **Nathan SS, Kalaivani K, Chung PG (2005).** The effect of azadirachtin and nucleopolyhedrovirus on midgut enzymatic profile of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). *Pesticide Biochem Physiol.* 83: 46-57.
57. **Nathan SS, Kalaivani K, Chung PG, Murugan K (2006).** Effect of neem limonoids on lactate dehydrogenase (LDH) of the rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) (Insecta: Lepidoptera: Pyralidae). *Chemosphere.* 62(8): 1388-1393.
58. **Nathan SS, Kalaivani K, Murugan K and Chung PG (2005).** The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenée) the rice leaf folder. *Pestic Biochem Physiol.* 81: 113-122.
59. **Naumann K and Isman MB (1996).** Toxicity of neem (*Azadirachta indica* A. Juss.) seed extracts to larval honeybees and estimation of dangers from field application. *American Bee Journal.* 136: 518-520.
60. **Nicoletti M, Petitto V, Gallo FR, Multari G, Federici E and Palazzino G (2012).** The modern analytical determination of botanicals and similar novel natural products by the HPTLC fingerprint approach. *Stud. Nat. Prod. Chem.* 37: 217–258.
61. **Norten E and Pütz J (1999).** Neem: India's miraculous healing plant. Rochester, VT: Inner Traditions/Bear & Co.
62. **Parangama PA, Connolly JD and Strang RHC (2003).** Effects of azadirachtin on incorporation of 35S-Cysteine into peptides in the brain and corpus cardiacum in locust schistocerca gregaria. *J Natn Sci Foundation.* 31: 459-471.
63. **Qiao J, Zou X, Lai D, Yan Y, Wang Q, Li W, et al. (2014).** Azadirachtin blocks the calcium channel and modulates the cholinergic miniature synaptic current in the central nervous system of *Drosophila*. *Pest Manag. Sci.* 70: 1041–1047.
64. **Raguraman S and Kannan M (2014).** Non-target effects of botanicals on beneficial arthropods with special reference to *Azadirachta indica*, In : *Advances in Plant Biopesticides*, ed. D. Singh (New Delhi: Springer), 173–205.
65. **Ram P, Vivek K and Kumar SP (2014).** Nanotechnology in sustainable agriculture: present concerns and future aspects. *Afr. J. Biotechnol.* 13, 705–713.
66. **Riyajan SA and Sakdapipanich JT (2009).** Encapsulated neem extract containing Azadirachtin-A within hydrolyzed



- polyvinyl acetate for controlling its release and photodegradation stability. *Chem. Eng. J.* 152: 591–597.
67. **Robertson SL, Ni W, Dhadialla TS, Nisbet AJ, McCusker C, et al (2007).** Identification of a putative azadirachtin binding complex from *Drosophila* Kc167 cells. *Arch Insect Biochem Physiol.* 64: 200-208.
  68. **Roychoudhury R (2016).** Neem products, in *Ecofriendly Pest Management for Food Security*, ed. Omkar (Amsterdam: Elsevier), 545–562. Sahayaraj K, Namasivayam SKR and Rath J M (2011). Compatibility of entomopathogenic fungi with extracts of plants and commercial botanicals. *Afr. J. Biotechnol.* 10: 933–938. Sarawaneeyaruk S, Krajangsang S and Pringsulaka O (2015). The effects of neem extract and azadirachtin on soil microorganisms. *J. Soil Sci. Plant Nutr.* 15 (4).
  69. Schmutterer H (1990). Properties and potential of natural pesticides from the neem tree *Azadirachta indica*. *Annu Rev Entomol.* 35: 271-297.
  70. **Scudeler EL and dos Santos DC (2013).** Effects of neem oil (*Azadirachta indica* A. Juss) on midgut cells of predatory larvae *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae). *Micron* 44: 125–132.
  71. **Scudeler EL, Garcia ASG, Padovani CR and Santos DC (2013).** Action of neem oil (*Azadirachta indica* A.Juss) on cocoon spinning in *Ceraeochrysa claveri* (Neuroptera: Chrysopidae). *Ecotoxicol. Environ. Saf.* 97: 176–182.
  72. **Scudeler EL, Padovani CR and dos Santos DC (2014).** Effects of neem oil (*Azadirachta indica* A. Juss) on the replacement of the midgut epithelium in the lacewing *Ceraeochrysa claveri* during larval-pupal metamorphosis. *Acta Histochem.* 116: 771–780.
  73. **Seljåsen R and Meadow R (2006).** Effects of neem on oviposition and egg and larval development of *Mamestra brassicae* L: dose response, residual activity, repellent effect and systemic activity in cabbage plants. *Crop Prot.* 25: 338–345.
  74. **Senthil Nathan S, Young Choi M, Yul Seo H, Hoon Paik C, Kalaivani K, et al. (2008).** Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown plant hopper *Nilaparvata lugens* (Stal). *Ecotoxicol Environ Saf.* 70: 244-250.
  75. **Senthil Nathan S, Kalaivani K, Sehoon K and Murugan K (2006).** The toxicity and behavioural effects of neem limonoids on *Cnaphalocrocis medinalis* (Guenée), the rice leaf folder. *Chemosphere* 62: 1381–1387.
  76. **Senthil-Nathan S, Choi MY, Seo HY, Paik CH and Kalaivani K (2009).** Toxicity and behavioral effect of 3β,24,25-trihydroxycycloartane and beddomei lactone on the rice leaf folder *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae). *Ecotoxicol. Environ. Saf.* 72: 1156–1162.
  77. **Servin AD and White JC (2016).** Nanotechnology in agriculture: next steps for understanding engineered nanoparticle exposure and risk. *Nanoimpact* 1: 9–12.
  78. **Seufert V, Ramankutty N and Foley JA (2012).** Comparing the yields of organic and conventional agriculture. *Nature.* 485: 229–232.
  79. **Sieglwart M, Graillot B, Blachere Lopez C, Besse S, Bardin M, Nicot PC, et al. (2015).** Resistance to bio-insecticides or how to enhance their sustainability: a review. *Front. Plant Sci.* 6: 381.
  80. **Sivakumar M and Gunasekaran K (2011).** Management of root knot nematodes in tomato, chilli and brinjal by neem oil formulations. *Journal of Biopesticides.* 4(2)
  81. **Srilatha B (2011).** Nanotechnology in agriculture. *J. Nanomed. Nanotechnol.* 2: 123.
  82. Subrahmanyam B, Muller T and Rembold H (1989). Inhibition of turnover of neurosecretion by azadirachtin in *Locusta migratoria*. *J Insect Physiol.* 35: 493-500.
  83. **Sukanya SL, Sudisha J, Hariprasad P, Niranjana SR, Prakash HS and Fathima SK (2009).** Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. *Afr. J. Biotechnol.* 8: 6677–6682.
  84. **Tangtrakulwanich K and Reddy GVP (2014).** Development of insect resistance to plant biopesticides: an overview, In : *Advances in Plant Biopesticides*, ed. D. Singh (New Delhi: Springer), 47–62.
  85. **Tavares WS, Costa MA, Cruz I, Silveira RD, Serrao JE and Zanuncio JC (2010).** Selective effects of natural and synthetic insecticides on mortality of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and its predator *Eriopsis connexa* (Coleoptera: Coccinellidae). *J. Environ. Sci. Health B* 45: 557–561.
  86. **Thackera JRM, Bryan WJ, McGinley C, Heritage S and Strange RHC (2003).** Field and laboratory studies on the effect of neem (*Azadirachta indica*) oil on the feeding activity of the lagre pine weevil (*Hylobius abietis* L.) and implications for pest control in commercial conifer plantations. *Crop protection.* 22: 753-760. Wang Y, McAllister TA, Yanke LJ and Cheeke PR (2000). Effect of steroidal saponin from *Yucca schidigera* extract on ruminal microbes. *J Appl Microbiol.* 88: 887-896.
  87. **Weathersbee AA and McKenzie CL (2005).** Effect of a neem biopesticide on repellency, mortality, oviposition, and development of *Diaphorina citri* (homoptera: psyllidae). *Fla. Entomol.* 88: 401–407. Wilps H, Kirkilionis E and Muschenich K (1992). The effects of neem oil and azadirachtin on mortality, flight activity, and energy metabolism of *Schistocerca gregaria* forskal—A comparison between laboratory and field locusts. *Comp. Biochem. Physiol. C Comp. Pharmacol.* 102: 67–71.
  88. **Xu J, Fan QJ, Yin ZQ, Li XT, Du YH, Jia RY, et al. (2010).** The preparation of neem oil microemulsion (*Azadirachta indica*) and the comparison of acaricidal time between neem oil microemulsion and other formulations in vitro. *Vet. Parasitol.* 169: 399–403.