एज़ाडायरेक्टा इंडिका ए. जस (एवं) *सिम्बोपोगोन नार्डस* (एल.) से उत्पन्न पादप रसायनों/तेल की नवोन्मेषी फार्मूलेशन (फार्मलूशनों) का विकास

DEVELOPMENT OF INNOVATIVE FORMULATION(S) OF PHYTOCHEMICALS/OIL DERIVED FROM *AZADIRACHTA INDICA* A. JUSS AND *CYMBOPOGON NARDUS* (L.)

EISA OSMAN MOHAMED ALI

DIVISION OF AGRICULTURAL CHEMICALS

ICAR- INDIAN AGRICULTURAL RESEARCH INSTITUTE

NEW DELHI – 110 012, INDIA

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By

EISA OSMAN MOHAMED ALI

A thesis

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Approved by:

Chairman: Dr. N. A. Shakil _____

Co-chairman: Dr. Rajesh Kumar _____

Members: Dr. Virendra Singh Rana _____

Dr. Chitra Srivastava _____

Dr. Pankaj _____

Dr. Lakshman Prasad _____

Division of Agricultural Chemicals
ICAR-Indian Agricultural Research Institute
New Delhi-110012, India

Dr. N. A. Shakil
Principal Scientist

CERTIFICATE

This is to certify that the thesis entitled "**Development of innovative formulation(s) of phytochemicals/oil derived from *Azadirachta indica* A. Juss and *Cymbopogon nardus* (L.)**" submitted to the Faculty of the Post-Graduate School, ICAR-Indian Agricultural Research Institute, New Delhi, in partial fulfillment of **Doctor of Philosophy** in **Agricultural Chemicals**, embodies the results of bonafide research work carried out by **Eisa Osman Mohamed Ali** under my guidance and supervision and that no part of this thesis has been submitted for any other degree or diploma. The assistance and help availed during the course of investigation as well as source of information have been duly acknowledged by him.

Place: New Delhi
Dated:

(Dr. N. A. Shakil)
Chairman
Advisory Committee

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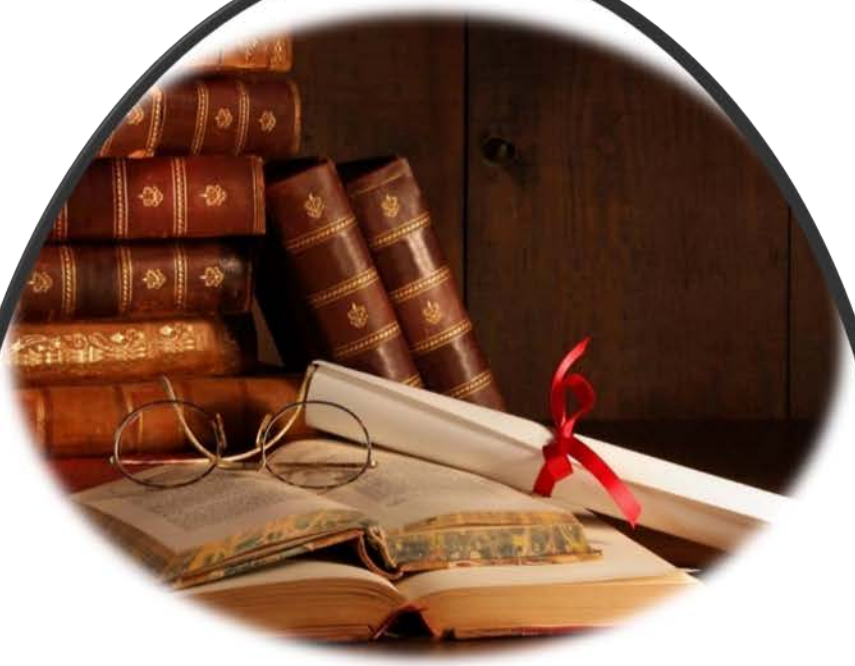
Dated:



Dedicated to My
Beloved Parents, Chairman,
My beloved wife Nedal
And my beloved children
(Nabil and Alla)

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1. INTRODUCTION

For global food security, the agricultural sector of the world economy must achieve a production level that ensures adequate food supply for increasing population. Worldwide, agriculture suffers from huge annual losses of crops due to insect-pests, diseases and weeds. The agricultural crop loss due to insect-pests, diseases, nematodes and weeds has been recorded even up to 50% in spite of the application of modern crop protection measures (Devakumar *et al.,* 2007). Of these, a considerable annual crop loss is due to plant diseases caused by phytopathogenic fungi, estimated up to about 20% and poses continuous challenge to modern agriculture disease management system. These phytopathogenic fungi infect seeds, seedlings and mature plants in the field causing many diseases such as collar rot, wilt, damping off and dry root rot (Aggarwal *et al.,* 2009). Among these fungi, *Rhizoctonia solani* and *Sclerotium rolfsii* infect many field crops. At present, quick and effective management of most of plant pathogen fungi is generally achieved by the use of synthetic fungicides. In recent years, a large number of synthetic fungicides are banned in western world because of their undesirable attributes such as acute toxicity, long degradation periods, accumulation in food chain and toxicity to non- targeted organisms (Gatto *et al.,* 2011).

Spodoptera litura (Fab.) (Noctuidae: Lepidoptera), commonly known as tobacco caterpillar, is an economically important polyphagous insect of seasonal crops in many countries including India, Japan, China and Southeast Asia (Sahayarij *et al*., 2007). It is responsible for huge yield losses in many economically important cultivated crops; sometimes causes up to 100% yield loss in field (Abbas *et al*., 2014). Various insecticides have been used for management of *S. litura*. However, due to their extensive use, this pest has developed multiple types of resistance, perhaps contributing to difficulties in controlling it in the field (Abbas *et al.,* 2012). Outbreak of this pest occurred during 1987-88 in Andhra Pradesh and Tamil Nadu, where it caused 66% loss in the yield of seed cotton running into a loss of few hundred crores of rupees (Dhaliwal and Arora, 2001).

The use of pesticides is indispensable for the production of high crop yields in agriculture, but there is a considerable public concern about the unintentional adverse impact of pesticides on non-target organisms and environmental quality mainly soil,

water and air (Mohamed, 2007). These problems compelled researchers to find out alternative control measures, such as botanicals, which are effective, safe, environment friendly and also reduce the dependence on synthetic fungicides. In addition to a wide group of secondary metabolites such as flavonoids, terpenoids, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (Dissanayake and Jayasinghe, 2013), essential oil, a complex mixture of organic compounds from plants are reported to have antibacterial, antifungal, antivirual, antiparasitic and antidermatophytic properties (Tabassum and Vidyasagar, 2013), which could be useful to develop effective biopesticide of plant origin.

Azadirachta indica A. Juss (Family: Meliaceae), commonly known as neem, is native of south eastern Asia, mainly cultivated in Indian sub-continent and used for the development of industrial products (Jerobin *et al.,* 2012). It possesses medicinal, antibacterial, antifungal and pest control properties and is a rich source of azadirachtin, an insect control agent (Lucantoni *et al.,* 2006; Ghotbi *et al.,* 2014; Chary, 2011). Several compounds have been reported from neem tree. Among these, azadirachtin-A, nimbin and salanin are the major triterpenoids having several biological activities (Biswas *et al.,* 2002; Subapriya and Nagini, 2005). Azadirachtin is widely used in agriculture to combat insects, nematodes, fungi and bacteria. However, because of its susceptibility towards light, temperature and also degradation by microorganisms, it is not stable for prolonged period (Khater, 2011). Efforts have been made in the past to stabilize azadirachtin-A, either through structural modification, such as reduction to dihydro- and tetera hydro-derivatives or by using stabilizers, including antioxidants and UV/sun screens (Sharma *et al*., 2006). Also, formulations of azadirachtin-A have been developed using capsules of sodium alginate, reticulated with glutaraldehyde and coated with natural rubber, encapsulation efficiency greater than 90% was achieved for azadirachtin (Riyajan and Sakdapipanich, 2009). Bioassays showed that the nanoparticles containing azadirachtin (5000 mg/kg) were effective in combating *Plutella xylostella*, with 100% mortality of the larvae (Shah *et al.,* 2016). Costa *et al.* (2014) prepared different types of formulations viz., nanocapsules, microparticles and emulsion concentrates containing azadirachtin. It was found that the nanocapsule formulation was more stable to degradation by UV radiation. The unencapsulated compound was completely

degraded within seven days, while the encapsulated azadirachtin showed only 20% degradation after 14 days.

Neem oil plays influential role as an effective natural pesticide having strong antifeedant and growth regulating properties (Chang *et al.,* 2005). The oil of the neem seeds (*A. indica* A. Juss) contains potentially bioactive compounds, which are effective in controlling pests of stored grains (Boeke *et al.,* 2004). Nanoemulsion of neem oil in Tween 20 was found as potent larvicidal for *Culex quinquefasciatus* (Anjali *et al.,* 2011). Okumu *et al.,* (2007) reported the emulsions of neem oil with emulsifier and isopropanol to be effective against *Anopheles gambiae.*

Cymbopogon nardus (L.) Rendle (Family: Poaceae), commonly known as citronella, is a perennial aromatic grass used for production of citronella oil besides *C. winterianus* Jowitt, which is used as mosquito repellent, household fumigant and in soaps and cosmetics. Previous studies revealed that citronella oil has antibacterial and antifungal activities. The main chemical constituents of *C. nardus* oil are citronellol, geranial, citronellal, citral and geranyl acetate (Nakahara *et al.,* 2003).

The US Environmental Protection Agency has registered some essential oils including Citronella, Lemongrass and Eucalyptus oils, as insect repellent ingredients suitable for application to skin (Nerio *et al.,* 2010). There are reports on the insect repellency from mint (*Teucrium leucocladum*), citronella (*Cymbopogon nardus*), basil (*Ocimum basilicum*) and their cultivars, thyme (*Thymus vulgaris* L.), neem (*Azadirachta indica* A. Juss), and lemongrass (*Cymbopogon citratus)* (Jaenson *et al.,* 2006). Previous studies using the disc diffusion method had revealed that citronella oil exhibited antibacterial and fungicidal activities (Nakahara *et al.,* 2003). Citronella oil has demonstrated good efficacy against mosquitoes with nontoxic action in concentrations ranging from 0.05% to 15% (w/v) alone or in combination with other natural or commercial insect repellent products (Fradin, 1998).

Nanoemulsions are known as isotropic, thermodynamically stable, transparent system of oil; water and surfactant with a droplet size usually in the range of 10-100 nm (Shafiq *et al.,* 2007; Mei *et al.,* 2003). Nanoemulsions have advantages over conventional emulsions due to small size, large surface area, slow release of active compounds and stability against sedimentation and other properties (Wang *et al.,* 2007). A study showed that no precipitation occurred in the nanoemulsion

formulation of β-cypermethrin on dilution in contrast to commercial micro emulsion with slower release (Zeng *et al.,* 2008). Similarly, nanoemulsion of neem oil showed increased larvicidal effect against *Culex quinquefasciatus* and LC_{50} decreased with droplet size, indicating an increased uptake of smaller droplets (Anjali *et al.,* 2011). Though, research on nanoemulsions is growing, very little work has been reported where plant oils have been utilized in crop protection due to their insolubility in water. This limitation can be overcome by encapsulating these oils in oil-in-water emulsions or nanoemulsions. (Chang *et al.,* 2012; Ziani *et al.,* 2011). It is reported that spontaneous emulsification of essential oils into nanoemulsion is simple and cost-effective method (Chang *et al.,* 2013). The bioactive essential oils which are volatile and sensitive to degradation are suitable for such nanoemulsions (Oliveira *et al*., 2014).

(The present work envisaged to develop and evaluate nanoemulsions based on neem and citronella oils using low energy spontaneous emulsification technique, which, to the best of our knowledge, is first report of its kind. The objectives of proposed work are as follows:

1. To develop nanoemulsions based on neem and citronella oils using low energy spontaneous emulsification technique.
2. To study the physio-chemical parameters of the developed formulations.
3. To study the fungicidal activity of the developed formulations of neem and citronella oil against *Rhizoctonia solani* and *Sclerotium rolfsii.*
4. To study the insect growth regulator activity of the developed formulations of neem and citronella oil against *Spodoptera litura* larvae.)

2. REVIEW OF LITERATURE

2.1 Phytopathogenic fungi and insect pests

For global food security, the agricultural sector of the world economy must achieve a production level that ensures adequate food supply to feed the increasing population. Also, productivity of crops grown for human consumption is at risk due to incidence of pests, especially weeds, pathogens and insect pests. According to the report of FAO, US $120 billion losses worldwide were caused by 20-40% decrease in crop yield, due to the attack from pathogenic organisms and insect-pests (Suresh *et al.*, 2017). Crops losses due to harmful organisms can be substantial and may be reduced through crop protection measures (Oerke, 2006). Insect pests and diseases are among the most important limiting factors that affect crop production, causing annual yield loss conservatively estimated at 50%. More than 70 different types of diseases caused by fungi, bacteria, viruses or nematodes have been reported (Singh *et al.*, 2015).

Rhizoctonia solani, a soil borne pathogenic fungi, is widely dispersed and invades a broad range of plants, including staple food crops such as rice, wheat and potato, as well as cash crops such as cotton and sugar beet and is a threat worldwide (Agrios, 2015; Woodhall *et al*., 2007). This fungus causes major losses to the production of important crops including wheat, rice, soybean, maize, potato, sugar beet and many more (Sneh, 1991). *R. solani* commonly causes disease by infecting roots and stems and inducing necrotic lesions from which plant obtains nutrients. Black scurf of potato caused by *R. solani* is a common and important disease of potato tubers in potato growing areas of the world. This disease affects potato development from emergence to harvest (Bokhari *et al.*, 2015).

Rice (*Oryza sativa*) is the staple food crop of over half of the world's population. Losses due to pests and diseases are one of the major constraints in rice production. Sheath blight and bacterial blight diseases of rice cause considerable loss, especially, in areas where high yielding varieties are grown (Kagale *et al*., 2004*). R. solani* is a major fungal pathogen of rice (*Oryza sativa* L.), and causes sheath blight that results great yield losses in all rice-growing regions of the world (Zheng *et al* 2013). It is a serious disease in all rice growing countries. It causes considerable yield

loss, accounts for 6 to 10% annually under high-input and high production environments in both temperate and tropical regions (Mew *et al.*, 2004).

Sheath blight of rice caused by *R. solani*, first reported from Japan, is emerging as a very destructive disease under favorable weather conditions in rice growing areas of the world which ultimately causes substantial yield losses (Ali *et al.*, 2015). The symptom in infected plants appears on the sheath and spreads to the leaves. The disease causes more than 50% economic loss (Suharti *et al.*, 2016). *R. solani* is best known to cause various plant diseases such as collar rot, root rot, damping off and wire stem.

In addition, *Sclerotium rolfsii*, soil-borne fungal pathogen also causes disease on a wide range of agricultural and horticultural crops such as sweet potato (*Ipomea batatas*), pumpkin (*Cucurbita pepo* L.), corn (*Zea mays*), wheat (*Triticum vulgare*) and peanut (*Arachis hypogea*) (Errakhi *et al.*, 2007). It can also cause a variety of disease in plants, including wilt and southern blight. It has a very extensive host range which includes more than 500 plants particularly in the tropical, subtropical and warm temperate areas (Shakil *et al.*, 2010). Disease caused by this pathogen leads to heavy losses in vegetable crops, especially during the wet season when weather conditions are favorable for both crop production and growth and dissemination of the sclerotia of the pathogen (Shakil *et al.*, 2010).

Synthetic fungicides are mainly used for effective management of phytopathogenic fungi in field. However, the chemical methods of disease management are expensive and can affect the beneficial microbial population present in the ecosystem. The obvious pollution problems due to indiscriminate use of synthetic pesticides and their toxic effect on non-target organisms have prompted investigations on exploiting pesticides of plant origin. Natural plant products are important sources of new agrochemicals for the control of plant diseases (Kagale, 2004). In recent years, a large number of synthetic fungicides have been banned in western world because of their undesirable attributes such as acute toxicity, long degradation periods, accumulation in food chain and toxicity to non- targeted organisms (Gatto *et al.*, 2011).

2.2 Insect pest

Spodoptera litura (Fab.) (Noctuidae: Lepidoptera), commonly known as tobacco caterpillar is an economically important polyphagous insect in many agricultural and horticultural crops in many countries including India, Japan, China and Southeast Asia (Sahayrij *et al*., 2007). It also has a wide range of host, which belongs to about 40 plant families. It may also cause loss of many economically important cultivated crops even up to 100% in field (Abbas *et al.,* 2014). In India, outbreak of this pest occurred during 1987-88 in Andhra Pradesh and Tamil Nadu, where it caused 66% loss in yield of cotton seed and also caused a loss of crores of rupees (Dhaliwal and Arora, 2001). It is reported to attack more than 200 different species of plants, of which 40 species are grown in India mainly, crucifers, cucurbits, groundnut, maize, castor, tea, tobacco, cotton, jute, lucerne, rice, soybean, cabbage, capsicum, potato including some ornamental plants (Abdul Razak *et al.,* 2014). In groundnut, 26-100% yield loss was reported due to *S. litura* (Maqsood *et al.,* 2016). Besides these, this insect pest was reported to cause 60% damage to a number of vegetable and other crops in India (Maqsood *et al.,* 2016). Various insecticides have been used for management of *S. litura*. However, due to their indiscriminate use, it has developed multiple types of resistance causing difficulties in controlling it in the field (Abbas *et al.,* 2012). It has shown resistance against almost all the insecticide groups such as organochlorines, carbamates, organophosphates (OPs) and pyrethroids (Kranthi *et al.,* 2002; Ahmad *et al.,* 2007) including new insecticides like lufenuron, fipronil, avermectins, indoxacarb, spinosad and insect growth regulators (Sudhakaran, 2002; Ahmad *et al.,* 2008).

Botanical insecticides are generally pest-specific and are relatively harmless to non-target organism including human. They are also biodegradable and harmless to the environment (Adeyemi, 2010). More than 2000 species of plants are known to possess some insecticidal properties (Klocke, 1989; Saxena, 1998) and some of them could be useful for development of effective and safe biopesticide for the management of agricultural insect-pests. Among the botanicals, essential oils are reported as rich source of bioactive compounds, including larvicides, repellents, insect growth regulators, antifeedants, ovicidal, oviposition, deterrents, and compounds that reduce fecundity and fertility (Zibaee *et al.,* 2016). Essential oils are complex mixtures of highly volatile chemicals that are typically derived from the steam

distillation of aromatic plant foliage and most of them are relatively non-toxic to mammals and fish in toxicological tests, and meet the criteria for “reduced risk” pesticides (Koul *et al.,* 2008). Although, the research interest on the insecticidal activity of plant essential oils has been gaining more attention recently, most of the studies still focus on simple screening of active plant source or identification of active compounds (Tak and Isman, 2016). Not much research efforts have been reported to develop formulations of these essential oils for achieving optimum activity against target pest in actual field condition.

In recent years, alternative to synthetic pesticides for effective pest control in different agro-systems has been investigated. Amongst them, plant-derived products have proved to be eco-friendly, residue free, biodegradable and cost effective. Utilization of these products drew attention of researchers and policy makers in developing and less developed countries (Gahukar, 2014). Plant biodiversity has provided an excellent source of biologically active substances (phytochemicals) that are produced by plants acquired for defense (Satya, 2007).

2.3 Neem (*Azadirachta indica)*

Among tree species, *A. indica* A. Juss (Family: Meliaceae), commonly known as neem, is a native of South Eastern Asia, mainly cultivated in Indian sub-continent and used for the development of industrial products (Jerobin *et al.,* 2012). It is one of the richest sources of secondary metabolites in nature. Neem tree is an attractive, broad-leafed evergreen that can grow up to 30 m in height, with spreading branches extending some 10 m. (Paulsen, 2010).

2.4 Medicinal properties of various parts of neem tree

Neem (*A. indica*) is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. It is cultivated on large scale for medical purpose and as raw materials for bio-pesticides in Africa, Latin America, Australia, India and China (Schmutterer, 2002). The medicinal utilities have been described, especially for leaf, fruit and bark (Biswas *et al.,* 2002). Neem is believed to possess antiseptic, anti-helminthic, anti-diabetic and antihypertensive properties (Darshan and Doreswamy, 2004). Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders,

constipation and also as a general health promoter (Biswas *et al.,* 2002). All parts of Neem tree are used in traditional Indian medicine as household remedy against various human diseases such as pain, fever and infection (Botelho, 2008). Research undertaken in the University of Nigeria showed the medicinal properties of fractionated acetone/water neem leaf extract (Anyaehie, 2009). Tests conducted at the King Institute of Preventive Medicine, Chennai in December 2012 found that the Siddha neem preparation brought down symptoms and hastened the recovery of patients affected by dengue (Narayan, 2012). Neem is also used in cancer (Mahapatra *et al*., 2011), skin disorders (Adel-ghaffer and Semmler, 2007), diabetes (Dixit *et al*., 1986), ulcers (Maity *et al.,* 2009), antibacterial (Dhayanithi *et al.,* 2010), antifungal (Wang *et al.,* 2010) and antiviral (Tiwari *et al*., 2010). The United Nation declared neem tree as tree of 21st century and the US National Academy of Science published a report in 1992 entitled Neem: a Tree for Solving Global Problem (Kumar and Navaratnam, 2013). Some of the medicinal attributes of various parts of neem have been summarized in (Table 2.1).

Table 2.1 Some medicinal uses of various parts of neem (Biswas *et al.,* 2002)

Part	**Medicinal use**
Leaf	Leprosy, eye problem, epistaxis, intestinal worms, anorexia, biliousness, skin ulcers
Bark	Analgesic, fever
Flower	Bile suppression, elimination of intestinal worms and phlegm
Fruit	Piles, intestinal worms, urinary disorder, epistaxis, phlegm, eye problem, diabetes, wounds and leprosy
Twig	Cough, asthma, piles, phantom tumour, intestinal worms, spermatorrhoea, obstinate urinary disorder, diabetes
Gum	Skin diseases (ringworms, scabies), wounds and ulcers
Seed pulp	Leprosy and intestinal worms
Oil	Leprosy and intestinal worms
Root, bark, leaf, flower and fruit together	Blood morbidity, biliary afflictions, itching, skin ulcer, burning sensation and leprosy

2.4.1. Antibacterial activity

Recent research shows the isolation and identification of the antibacterial active compounds from petroleum ether extract of neem oil (Zhang *et al*., 2010). Pu *et al.,* (*2*010) showed antifungal activity of 9-octadecanoic acid- hexadecanoic acid-tetrahydrofuran-3, 4 diyl ester from neem oil. Elavarasu *et al.,* (2012), studied *in vitro* anti-plaque activity of neem oil. Experiment was conducted to evaluate the antibacterial activity of the bark, leaf, seed and fruit extracts of *A. indica* (neem) on bacteria isolated from adult mouth and results revealed that bark and leaf extracts showed antibacterial activity against all the test bacteria. Furthermore, seed and fruit extracts showed antibacterial activity only at higher concentrations (Singhal and Bhatt, 2016).

Azadirachtin has been shown to possess different biological activity such as antibacterial, antimalarial and antifungal (Ghotbi *et al.,* 2014). The oil from the neem leaves is recognized to possess antibacterial activity against a wide spectrum of Gram-negative and Gram-positive microorganisms, including *Mycobacterium tuberculosis* and S*treptomycin*-resistant strains (Subapriya and Nagini, 2005).

2.4.2 Antifungal activity

Antimycotic activity of the extracts of different parts of neem has been reported (Akhila and Rani, 1999). Extracts of neem leaf are effective against certain human fungi, including *Trichophyton, Epidermophyton, Microsporum, Trichosporon, Geotricum and Candida* (Khan *et al.,* 1987). The antifungal activity of neem has been attributed to volatile sulfides, limonoid and gedumin (Iyer, 1991).

2.4.3 Antiviral activity

Galhardi *et al.,* (2012) studied *in vitro* antiviral property of *A. indica* polysaccharides against polio virus. Saha *et al.,* (2010) showed water extracted polysaccharides from *A. indica* leaves with anti-bovine herpes virus type 1 (BoHV-1) activity. The research of Xu *et al.,* (2012) showed the *in vitro* antiviral activity of neem seed kernel extracts against duck plague virus. Tiwari *et al.,* (2014) showed *in vitro* antiviral activity of neem (*A. indica* L.) bark extract against herpes simplex virus type-1 infection.

2.4.4 Anticancer activity

Chatterjee *et al.,* (2010) identified sulfonoquinovosyl diacylglyceride (SQDG) from *A. indica* and conducted studies on its cytotoxic activity and DNA binding properties. A review of the anticancer activity of *A. indica* was carried out by Paul *et al.,* (2011). Veeraraghavan *et al.,* (2011) showed the effect of neem leaf extract on protein-regulated cell death/ radio sensitization in pancreatic cancer cells. Mahapatra *et al.,* (2011) showed novel molecular targets of *A. indica* associated with inhibition of tumor growth in prostate cancer.

2.4.5 Skin diseases

Neem has an effect on chronic skin diseases such as acne, psoriasis, eczema, ringworm and even stubborn warts which can clear up easily when high quality, neem oil is used. Neem oil and leaves has been used in Siddha medicine for the treatment of skin diseases (Thas and Siddha, 2008). In addition, neem oil can be used as an excellent component of cosmetics to help clear, beautify and rejuvenate the skin.

2.4.6 Antiparasitic agents

Historically, neem has been used to rid the body of all forms of parasites. Neem quickly kills external and internal parasites. Neem extracts have hormone mimics that interfere with the life cycle of parasites, inhibit their ability to feed and prevent the eggs from hatching (Kumar and Navaratnam, 2013). Abdel-Ghaffar *et al*., (2012) studied the efficacy of a single treatment of head lice with a neem seed extract.

2.4.7 Antimalarial agent

Neem extract and neem powder have been used by the urban people of African country as mosquito repellent (Karunamoorthi *et al.,* 2009). Neem leaf slurries can also be used as sustainable method to reduce the larvae density of malaria vector, *Anopheles gambiae* (Luong *et al.,* 2012). Experiment was conducted to evaluate the antimalarial activity of extracts using *Plasmodium berghei* infected albino mice and results revealed that neem leaf and stem bark extracts reduced the level of parasitemia in infected mice by about 51-80% and 56-87%, respectively (Akin *et al.,* 2013). Some other studies showed that azadirachtin and other limonoids available in neem extracts are active on malaria vectors (Nathan *et al*., 2005).

2.5 Pest control properties of neem

The compounds from neem have been reported to be effective bioinsecticides (Chary, 2011) and found useful in controling of nearly 400 insects of medical and veterinary species and flies, *Amblyomma variegatum*, lice, head louse, spiders, mosquitoes, cockroaches and fleas (Abdel-Ghaffar *et al*., 2012). All these attributes make neem the most promising botanical pesticide. It is environment friendly and controls insect pests primarily through physiological or behavioral effects. It is poor knockdown agent and takes long time to reveal its effect. This is often a disadvantage in a large scale adoption of neem pesticides.

Azadirachtin, a complex tetranortri-terpenoid limonoid from neem plant, is the main component responsible for antifeedant, repellent properties, growth disruptant effect and adverse effect on reproduction against insects (Roy and Gurusubramanian, 2011). It possesses antibacterial, antifungal and pest control properties (Lucantoni *et al*., 2006; Ghotbi *et al.,* 2014; Chary, 2011). Azadirachtin is considered non toxic to mammals, fish and pollinators, having low mammalian toxicity with LD50 >5000 mg/kg for rat and is classified by Environment Protection Agency (EPA) as class IV (Dubey *et al.,* 2010). Azadirachtin is widely used in agriculture to combat insects, nematodes, fungi and bacteria. However, because of its sensitivity towards light, temperature and also degradation by microorganisms, it is not stable for prolonged period (Khater, 2011). Efforts have been made in the past to stabilize it, either through structural modification such as reduction to dihydro- and tetra hydro- derivatives or by using stabilizers, including antioxidants and UV/sun screens (Sharma *et al.,* 2006).

2.5.1 Antifeedant activity

Limonoids (like azadirachtin, salanin and toosendanin from family Meliaceae and limonin from Rutaceae), clerodane diterpenoides, saponins, withanolides, quassinoids, sesquiterpenes, monoterpenes coumarins and isoflavonoids are well studied for their antifeedant activity (Parmar and Walia, 2001; Isman, 2006; Szczepanik *et al.,* 2005). Azadirachtin has both antifeedant and insect growth inhibiting properties. The research of Vattikonda and Sangam, (2016) showed that azadirachtin from *A. indica* has potential antifeedant activity against *Papilio demoleus* L. This compound can affect about 200 species of insects by acting as antifeedant and growth disruptor (Khalil, 2013).

2.5.2 Insect growth regulator activity

Neem has strong insect antifeedant and growth regulating substances (Wu *et al.,* 2006). Neem extracts have shown effect on regulation of growth as well as repellency (Tiwari *et al.,* 2014). Insect growth regulatory effects were seen in some other ingredients of neem seed kernels, namely 22-23-dihydro-23{3-methoxyazadirachtin, 3- tigloylazadirachtol, and 1-tigloyl-3-acetyl-l l-mcthoxyazadirachtin. Insect growth regulatory activity of azadirachtin is supposed to be due to its interaction with neuro-endocrine system of insect (Mordue *et al.,* 2005). At the physiological level, azadirachtin blocks the synthesis and release of molting hormones (ecdysteroids) from the prothoracic gland leading to incomplete ecdysis in mature insects (Franck *et al.,* 2009). In adult female insect, a similar mechanism of action leads to sterility (Isman, 2006).

2.5.3 Repellent activity

Repellents are substances that act locally or at a distance, deterring an arthropod from flying to, landing on or biting human or animal skin (or a surface in general) (Choochote *et al.,* 2007). Neem seeds formulations have shown antifeedancy, fecundity suppression, ovicidal, larvicidal activity, growth regulatory and repellent activity against a number of arthropods, at very low dosages (Egho, 2012; Benelli *et al*., 2015). Azadirachtin is the main component responsible for antifeedant, repellent properties and growth disruptant effect and adverse effect on reproduction against insects (Roy and Gurusubramanian, 2011).

2.5.4 Antifungal activity

Neem has demonstrated antifungal activity against fungi that cause certain plant diseases viz., rots, smuts**,** wilts, mildews, diebacks, blights and other plant diseases (Singh *et al.,* 2015). Its extract is one of the most important plant products which inhibit mycotoxin production. The main chemicals of neem oil with antifungal activities are a mixture of triterpenoidal and tetranortriterpenoid compounds. Neem leaf constituents are known to potentially inhibit aflatoxin production in *Aspergillus parasiticus* without affecting fungal growth (Ghorbanian *et al.,* 2008). Studies on the effect of neem extracts on growth, sporulation, and morphology and OTA production by *Penicillium verrucosum* and *P. brevicompactum* were investigated. The results show that neem extracts have

fungitoxic activity, even though their mode of action is still not fully understood (Mossini *et al.,* 2009). Nimbin also demonstrated antifungal activity by inhibiting the growth of *Tinea rubrum* (Biswas *et al.,* 2002).

2.6 Different chemical compounds of neem

Neem contains a vast array of biologically active compounds that are chemically diverse and structurally variable with more than 140 compounds isolated from different parts of the tree (Subapriya and Nagini, 2005). The compounds isolated from neem have been divided in to two major classes: isoprenoids and nonisoprenoids. The isoprenoids include diterpenoids and triterpenoids containing protomeliacins, liminoids, azadrone, and its derivatives, gedunin, vilasinin type of compounds and C- secomeliacins as nimbin, salanin, and azadirachtin. The non isoprenoids include proteins and carbohydrates, sulphur compounds, polyphenolics such as flavonoids and their glycoside, dihydrochalcone, coumarin, tannin, aliphatic compounds etc. Azadirachtin, 6-deacetyl-nimbin, azadiradione, nimbin, salanin and epoxyazadiradione were the major compounds obtained from neem.

Azadirachtin (0.068% w/v) is present in very low concentration (Anjali *et al*., 2011). Other limonids such as, salannol, gedunin, nimbolide, azadiradione, azadirachtol etc. which exert toxic activities are being investigated for their effectiveness (Schmutterer, 2002). Chemical structures of some of the bioactive compounds are presented in Figures-2.1, 2.2 and 2.3.

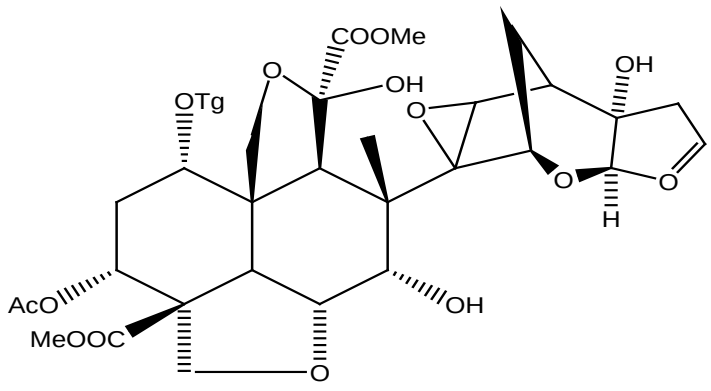


Figure-2.1 Structure of Azadirachtin

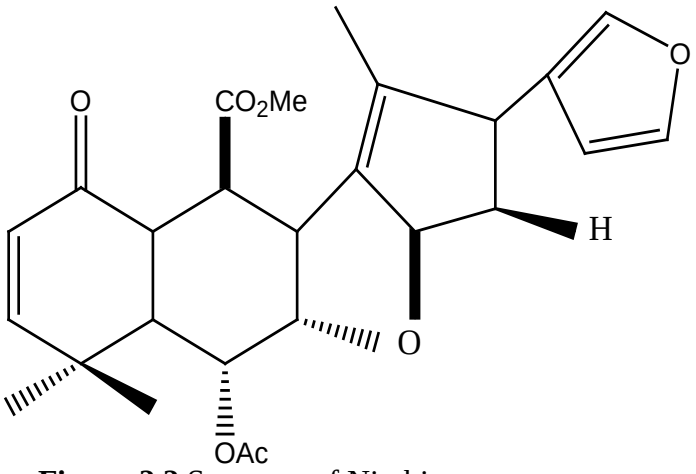


Figure-2.2 Structure of Nimbin

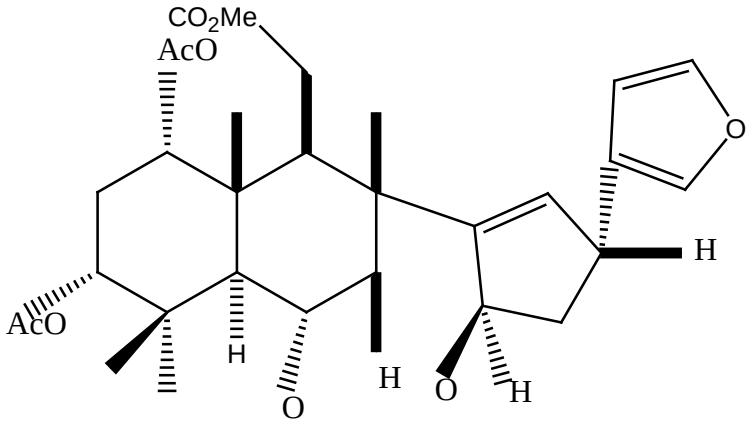


Figure-2.3 Structure of Salanin

2.7 Cymbopogon

Cymbopogon **(**Poaceae) is a genus comprising about 140 species, of which 45 species were reported from India **(**Rana *et al.,* 2016**).** *Cymbopogon sp*. occurs in tropical and subtropical regions of Asia, Africa, and America, from hilly areas to arid zones. Some species of the *Cymbopogon* genus, viz., *C. flexuosus, C. winterianus, C. martini var. motia, C. martini var. Sofia,* and *C. nardus var. nardus,* are used as the source of lemongrass oil, citronella oil, and palmarosa oil (Kumar *et al.,* 2009). It is an aromatic plant which can potentially control pathogens causing plant disease and/or induced resistance in plants (Maia *et al.,* 2014). It is cultivated for commercial production of an essential oil; which has huge demand in perfumery, cosmetics, soaps, toiletries, tobacco, and other products (Agarwal, 2008). *Cymbopogon* plants

have been traditionally used to repel mosquitoes in jungle regions such as the Bolivian Amazon (Moore *et al.,* 2007). This genus produces the most used natural repellents in the world (Trongtokit *et al.,* 2005).

C. nardus Randle is a perennial grass cultivated in Southeast Asia. The essential oil from *C. nardus* is known as citronella oil, traditionally used as mosquito repellent, household fumigant, or fragrance agent in food commodities, soaps. Citronella oil has an acaricidal (De Mello *et al*., 2014) and repellent effect (Labinas and Crocomo, 2002). Acaricidal properties of this oil are attributed to the presence of volatile substances such as citronellal, geraniol and citronellol (Almeid, 2011). Previous studies also revealed that citronella oil has antibacterial and antifungal activities (Nakahara *et al.,* 2003; Nakahara *et al.,* 2003).

The compositions of essential oil are affected by several factors such as cultivation and isolation technique. The most common components are citronellal (42.0%) and geraniol (21.0%) and other terpenes as shown in Figures-2.4 and 2.5. Tests done in tree nurseries showed that citronellal oil provided weed control without causing adverse effects on dormant broadleaf trees (Franck *et al*., 2009). The US Environmental Protection Agency has registered some essential oils including Citronella oil, Lemon grass, and Eucalyptus oils, as insect repellents suitable for application to skin (Nerio *et al.,* 2010).

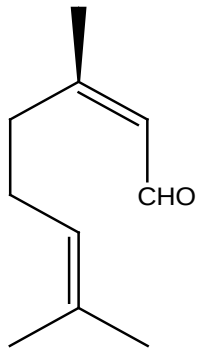


Figure-2.4 Citronellal

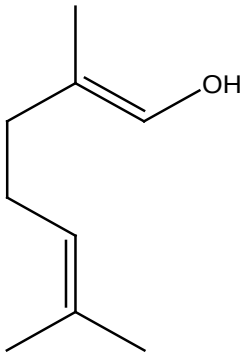


Figure-2.5 Geraniol

2.8 Nanotechnology

Pesticides are used in different ways based on physical and chemical characteristics. Due to several degradation processes such as leaching or destruction by light, temperature, microorganisms or water (hydrolysis), a small amount of these chemicals reaches the target site. For these reasons, repeated application becomes necessary for efficient control of target pests, which increase the cost and cause undesirable consequences to ecosystem and human health (Gavrilescu, 2005). Formulation technology tries to overcome such problems, however with variable success; nanotechnology is one promising way to do so efficiently.

Nanotechnology is the design, characterization, production and application of structures, devices and systems by controlling shape and size at nanometer scale (Murphy, 2008). Nanotechnology is a recent scientific approach that includes materials and equipments capable of manipulating physical as well as chemical properties of a substance at molecular levels (Rohini *et al.*, 2015). Nanotechnological innovations have the potential to create revolution in agricultural practices (Scott and Chen, 2002). International Organization for Standardization (ISO) materials with external dimensions or internal structure on nanoscle is referred to a nanomaterials and the term nanoscale is generally limited to about10- 100 nm.

Developing nanoformulations of materials allows a sufficient increase in water solubility, dissolution rate, dispersion uniformity upon application and increase the surface area (Muller and Keck, 2004). Insecticides, sensitive to light and other environmental degradation factors, may benefit from encapsulation into porous silica nanoparticles. Many essential oils have insecticidal activity but oils are volatile and

sensitive to degradation, and thus suitable for nanoencapsulation to overcome these disadvantages (Oliveria *et al.,* 2014). Nanoformulations are also used extensively in the area of pharmaceutical and personal care products (Anton and Vandamme, 2011). Numerous applications of nano materials have been found, in electronics, energy, textiles, pharmaceuticals, cosmetics, drug delivery systems and biomedicines (Table 2.2). This trend is flourishing rapidly with the promise that nanotechnology can provide possible solutions to almost everything, such as improved performance and new functionalities along with a great reduction in the use of resources and the waste generation. Therefore, nano materials are generally believed to increase profitability and sustainability over the conventional materials.

The principle objectives of nanopesticides are to increase solubility of poorly soluble active ingredient (a.i.) or it slow release or protect it from degradation, with better efficacy and low dose (Kah *et al*., 2013). The most common pesticides formulations for poorly water soluble active ingredient are emulsfiable concentrates (ECs) and oil-in water emulsions (O/Ws). The main disadvantages of ECs are the use of organic solvent leading to increase in the cost and flammability as well as dermal toxicity for the handlers and, most important of all, relatively poor stability after dilution (Knowles, 2005). O/W emulsions have been proposed as alternative to ECs. O/Ws generally consist of a mixture of surfactant, block polymer and polymeric surfactants. The drawback of O/W emulsions is that emulsification required a high energy input with particle size ranging from 500 nm to 2µm (Knowles, 2005). Most nanoformulations aiming to increase the solubility of a.i. are derived from these two mentioned approaches.

Table 2.2 Other applications of nanotechnology (Hubbe *et al.*, 2008)

Nanotechnologies	**Example**	**Potential applications**
Energy storage, production and conversion.	Novel hydrogen storage based on carbon nanotubes. Carbon nanotubes in composite film coatings for solar cells	Cheaper, clean energy improved rechargeable batteries.
Agriculture	Nanoporous zeolites for slow release and efficient delivery of water and fertilizers for plants and nutrients and drugs (nanovaccines) for livestock. Nanocapsules for herbicide delivery Nanosensors for soil quality and plant health monitoring	More efficient and sustainable production that requires fewer inputs.
Food processing and storage	Nanocomposites in plastic film for food packaging. Antimicrobial nanoemulsions for decontamination of food. Nanotechnology based antigen detection of contaminants.	Cheaper, safe food products with longer storage life.
Vector and pest detection and control	Nanosensors for pest and pathogen detection. Nanoparticles for new pesticides, insecticides and insect repellents.	More rapid development of safer control strategies with reduced losses.

The application of nanotechnology in pest management is slowly increasing. Specific application of nanotechnology in agriculture sector includes nano fertilizers, nano sensors, nano based treatment of agricultural wastes, nano pesticides etc. (Figure-2.6). Different types of formulations have been found in literature such as emulsions (especially nanoemulsions), polymeric nanocapsules and engineered nanoparticles containing product *viz*., nanoclays, metals, metal oxides etc (Kookana *et al*., 2014, Table 3).

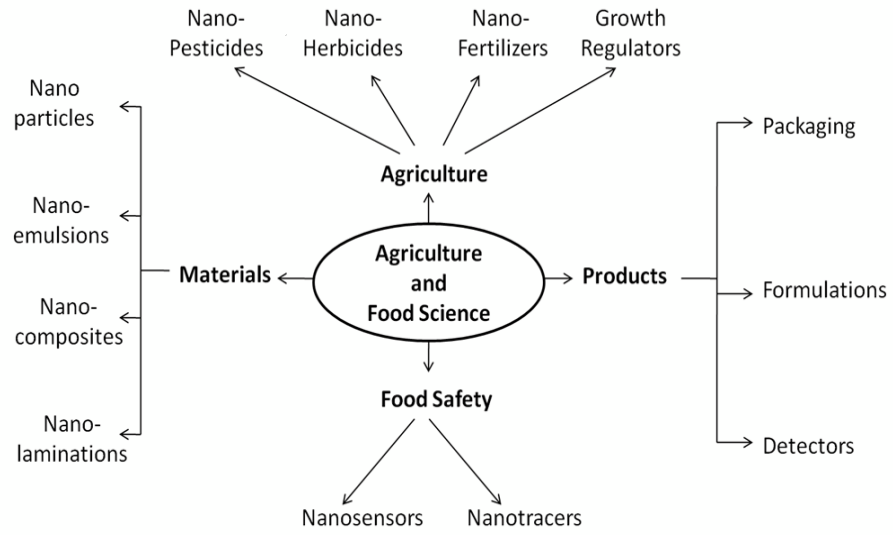


Figure-2.6 Applications of nanotechnology in agriculture

Table 2.3 Various nanoformulations reported in literature

Nanoformulation	**Primarily aiming**	**Reference**
Microemulsion	Increasing solubility	Katagi, 2008
	Increased or decreased sorption of the a.i. depending on concentration and type of surfactant (literature review)	
	Slower or faster degradation depending on a.i. and type of surfactant	Katagi, 2008
Nanoemulsion	Reduced hydrolysis (by up to 35%)/ triazophos technical grade.	Song *et al*., 2009
	Reduced volatilization (stable over 5 months storage)/ free garlic essential oil.	Yang *et al*., 2009
	Slower release (60 min)/ beta-cypermethrin EC (10 min)	Zeng *et al*., 2008
Polymer-based	Controlled release	Salma *et al*., 2010
	Reduced leaching from treated wood (2 up to 6-fold less concentrated leachates)/ aqueous solution of tebuconazole	
	Enhanced penetration in plants/ classical suspension of ethiprole (demonstrated	Boehm *et al*., 2003

	indirectly through the comparison of contact and systemic efficacy).	
	Release of a.i. can be adjusted by changing the proportions and molecular weight of the polymers.	Shakil *et al*., 2010; Sarkar *et al.*, 2012; Pankaj *et al.*, 2012; Kaushik *et al*., 2013; Koli, *et al*., 2014 and Majumder *et a*l., 2016
Solid lipid nanoparticle	Lower evaporation (after 48 hr, cumulative loss by evaporation was reduced by half)/ emulsion of *Artemisia arborescens* L essential oil.	Lai *et al*., 2006
Porous hollow Silica nanoparticles	Slower degradation due to UV-shielding (20% a.i. Remaining after 720 min)/ free a.i. and similar formulation using SiO_2 nanoparticles as carrier for avermectin (complete photodegradtion within 120 min).	Liu *et al*., 2006
	Slow release following first order, second order, power equation, or multistage pattern kinetic and influenced by pH, temperature.	Bin Hussein *et al*., 2002; Park *et al*., 2010; Qui *et al*., 2009
Layered double hydroxides and clays	Prolonged persistence (e.g., DT_{50} of cinnamic acid in soil were 6 days for an aqueous solution and 17 days when formulated with layer double hydroxides) similar bioavailability in soil (mineralization rate) /free atrazine.	El-Nahhal *et al*., 1999; Maqueda *et al*., 2009; Park *et al*., 2010
	Reduced leaching (up to 5 times smaller total amount leached) but similar persistence for an organo clay formulation of diuron/commercial formulation.	Trigo *et al*., 2010 Trigo *et al*., 2009
Metal and organic a.i.	Similar half-life in plant and soil / suspension concentrates of chlorfenapyr.	Cao *et al*., 2005
	Faster degradation / suspension concentrate of imidachlorprid in soil (half-lives of 2.8 and 6.2 days, respectively) and in soya bean plants (1.9–4.5 days).	Guan *et al*., 2010

Nanopesticides were primarily classified on the basis of their intended use, with the view of analyzing the possible consequences which are affecting their environmental fate. Sub-categories of nanopesticides were further classified which are based on the quantities and type of adjuvants sand expected discrepancies in terms of environmental fate (Figure-2.7).

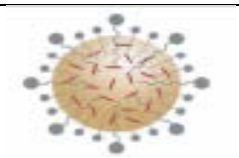
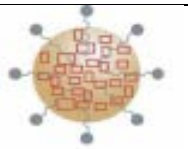
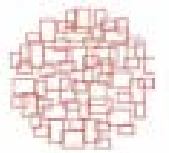
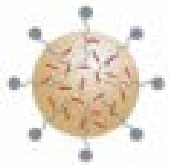
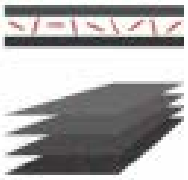
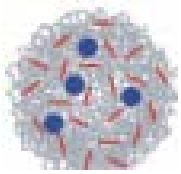
<table>
<tr><td colspan="4">Increase the solubility of poorly water-soluble (a.i.)</td></tr>
<tr><td>Micro-emulsion
(6-50 nm)</td><td>Nano-emulsion
(20-200 nm)</td><td colspan="2">Nano-dipersion
(50-200 nm)</td></tr>
<tr><td>  </td><td>  </td><td colspan="2">  </td></tr>
<tr><td colspan="4">Slow/targeted release and protection against premature degradation</td></tr>
<tr><td>Soft matrix</td><td colspan="3">Hard matrix</td></tr>
<tr><td>Polymer-based
(10-300 nm)</td><td>Solid lipid
(200 nm-100 µm)</td><td>Porous hollow silica
(100-200 nm)</td><td>L LDH and clays
(µm range)</td></tr>
<tr><td>  </td><td>  </td><td>  </td><td>  </td></tr>
<tr><td colspan="4">Containing nano -metal or oxides</td></tr>
<tr><td colspan="2">Associated with another (a.i)
(µm range)</td><td colspan="2">Alone
(1-30 nm)</td></tr>
<tr><td colspan="2">  </td><td colspan="2">  </td></tr>
</table>

Figure-2.7 Overview of nanopesticides (Kah *et al.*, 2013)

Recently, nanogels have been proposed for use in plant protection products as possible way to meet organic farming standards. Bhagat *et al.*, (2013) proposed the immobilization of pheromones within nanogel. Evaporation of pheromones in the nanogel was significantly reduced compared to the evaporation of the pure a.i, extending their effectiveness for up to 33 weeks compared to only three weeks for the pure a. i. The efficacy of nanogel formulation of essential oil extracted from *Lippia sidoides* was also shown to be superior to that of free oil (Bhagat *et al.*, 2013). Brunel

et al., (2013) proposed the use of pure chitosan to improve the performance of antifungal treatment based on copper. Many recent work using polymer based nanoformulations have shared the common objective of developing less harmful plant products through the use of biodegradable polymer.

Table 2.4 Efficacy comparison of nanoformulations with conventional formulations

Nanoformulation	**Efficacy**	**Reference**
Nanoemulsion	Permethrin nanoemulsion was found to be more efficient as larvicide	(Anjali *et al*., 2011)
	The efficacy of nanoemulsions of permethrin was significantly higher than that of the pure a.i., which again interpreted as indicating an increasing uptake of the nanoemulsions a.i.	(Kumar *et al.*, 2013)
	Garlic essential oil nanoemulsion was better than free garlic oil against *T. castaneum*	(Yang *et al*., 2009)
	β-cypermethrin nanoemulsion showed higher stability and increased bioefficacy.	(Wang *et al*., 2007)
	Nanoemulsion of neem oil showed Increased larvicidal effect against *Culex quinquefasciatus* and LC_{50} decreased with droplet size, indicating an increased uptake of smaller droplets.	(Anjali *et al.*, 2011)
	Slightly slower release of β- cypermethrin from nanoemulsion than from a commercialal EC.	(Zeng *et al*., 2008)
Nanodispersion	Nanoparticles containing azadirachtin (5000 mg/kg) were effective in combating *Plutella xylostella,* with 100% mortality of the larvae.	Shah *et al*., 2016)
Polymer based	Polymer based tebuconazole nanoformulation showed higher efficacy possibly inhibiting development of fungi to greater extent.	(Salma *et al*., 2010)
	Formulations of azadirachtin developed using capsules of sodium alginate, reticulated with glutaraldehyde and coated	(Riyajan and Sakdapipanich, 2009)

	with natural rubber. Encapsulation efficiency greater than 90% was achieved for azadirachtin.	
	Nanocapsules, microparticles and emulsion concentrates containing azadirachtin were found more stable to degradation by UV Radiation.	Costa, *et al*. (2014)
Nanometal + *a.i.*	Imidacloprid when used along with nanometal showed better toxicity against adult stage of *Martianus dermestoides*, as compared to the aqueous formulations.	(Guan *et al*., 2008)

Various types of nanoformulations, attempted to achieve specific characteristics of pesticides are listed in Table 2.5.

Table 2.5 Potential applications of nanotechnology in pesticides sector (Kookana *et al*., 2014)

Function	**How this can be achieved**	**Current examples**
Enhanced apparent solubility	Nano- and microemulsion	Emulsion-based registered pesticides, banner MAXX of Syngenta
Faster decomposition in soil/plant	Nanocatalyst- conjugated a.i in microcapsules	SDS-modified TiO_2/Ag conjugates with a.i such as Imidacloprid.
Targeted delivery	Nanocapsule	Nanoencapsulted glyphosate.
Protection against degradation	Nanocapsule	TiO_2-M262 polymer netaflumizone
Enhanced uptake	Nano- and microemulsion	Nanopermethrin; nanosphere insecticides
Enhanced toxicity to target organisms	Nanodispesion and nanosuspension	Nanodispersed triclosan
Nanoparticles as a.i	Nanomaterials and nanoclay	Registred nano-Ag biocides; Nano-Si.

2.9 Nanoemulsions

Nanoemulsions are known as isotropic, thermodynamically stable, transparent system of oil; water and surfactant with a droplet size usually in the range of 10-100 nm (Shafiq *et al.,* 2007; Mei *et al.,* 2003). Enormous interest in nanoemulsion is triggered by the wide range of applications in the pharmaceutical, cosmetic, food and chemical (Solons and Sole, 2012). Nanoemulsions can be developed by a number of different techniques, which are usually categorized as either high energy or low energy methods (McClements and Roa, 2011). The high energy emulsification system makes use of mechanical energy using homogenizer, shear stirring, high pressure homogenizers or ulter sound generators, whereas low energy emulsification methods use the chemical energy stored in the components of the system (Forgiarini *et al.,* 2001). Low energy methods mainly rely on the spontaneous formation of droplets at the boundary between oil and water phases and depend strongly on the nature of any surface of active molecules present, e.g., their solubility and molecular geometry. A number of different low energy approaches have been developed to form nanoemulsions, including spontaneous emulsification, phase inversion temperature (PIT), and phase inversion composition (PIC) methods (McClement and Roa, 2011)**.**

Nanoemulsions contain lower concentration of surfactant (5-10%) than microemulsion (about 20%). The nanoemulsion have distinct properties such as higher efficacy (Anjali *et al*., 2011), reduced hydrolysis (Song *et al.,* 2009), reduced volatilization of a.i. (Yang *et al*., 2009) as compared to other formulations, e.g., ECs. There are advantages of microemulsions which include improved tank mix, compatibility and low flammability due to low solvent contents in a continues water phase (Knowles, 2005). However, they suffer from disadvangtes such as low a.i. content (< 30%), high concentrations of surfactant (20%) (Lawrence, 2000), limited number of suitable surfactant system and handler toxicity issues (Knowles, 2005). Pesticide formulations contain various solvents made mostly from petroleum and there is a concern about using such solvents in pesticide formulations. The use of such solvents demands large amounts of surfactants and other additives which makes the cost of the formulations high. Hence, there is a need to develop a formulation which is simple, safe and cost effective to provide stable and efficacious products. Consequently, there is a need to identify ways and methods to enhance the efficacy of botanical extracts for their wide spread acceptance as a pesticide. Recently,

nanoemulsions are emergening as a promising tool for delivery system (Anjali *et al.*, 2011).

Most botanicals are readily biodegradable and are reportedly devoid of the various harmful effects associated with the use of synthetics (Ghosh *et al.*, 2012). The use of botanical pesticides including plant oils is increasing for protection of agricultural crops from insect-pests and diseases. However their use in agriculture faces a major drawback of low water solubility and volability. This limitation can be overcome by encapsulating these oils in oil-in-water (O/W) emulsions or nanoemulsions (Ziani, 2011; Chang, 2012). Nanoemulsions with plant based oils are finding growing interest due to its bioavailability and biocompatibility. Nanoemulsion based delivery systems have previously been used to encapsulate various kinds of lipophilic bioactive components, including antitumor agents, anti-inflammatory agents, vitamins and antimicrobials. Though, research on nanoemulsions is growing, very little work has been reported where plant oils have been utilized in crop protection due to their insolubility in water. It is reported that spontaneous emulsification of essential oils into nanoemulsion is simple and cost-effective method (Chang *et al.,* 2013). The bioactive essential oils which are volatile and sensitive to degradation are suitable for such nanoemulsions (Oliveira *et al.*, 2014).

In the past decades, new techniques, with an aim to improve the performance of pesticides, are being used by chemical industries and plant protection researchers through nanotechnological interventions. Encapsulation of active ingredients and reducing the particle size of the formulation are the key areas in which a group of researchers are actively working for developing innovative formulations. (Following are the potential applications of nanotechnology in agriculture:

1. Enhancement in the bio-efficacy of synthetic and natural pesticides.
2. Possible solutions of problems associated with plant protection products such as photodegradation, lower efficacy etc.
3. Reduced risk to environment and non target organisms.
4. Ensuring better handling safety to the user by coating of active ingredient in nano matrices.)

3. MATERIALS AND METHOS

3.1. Materials

Neem and citronella oils were purchased from local market. Tween 20, Triton-X 100 and Potato Dextrose Agar (PDA) were obtained from Himedia Labs, India. Distilled and standard hard water D. All the chemicals used were of analytical grades.

3.2 Instrument

Particle size analyzer : Microtrac (ZetatracTM) particle size and Zeta Potential analyzer.

Transmission Electron Microscope : TEM-JEOL1011, USA.

Magnetic stirrer : Labman™ digital magnetic stirrer with Temperature control (SUNSIM, India.)

Mechanical stirrer : (RQ-123, RPM 4000, REMI Motors, Mumbai-53, India).

Fourier transforms Infrared Spectrometer : Bruker Alpha-E sample.

Other instruments used were, sonication chamber, centrifuge, pH meter, constant temperature bath, Biochemical Oxygen Demand (BOD) Incubator, autoclave, laminar flow hood hot plate etc.

3.3 Glass and plastic ware

Conical flasks, plastic beakers, magnetic beads, glass stopped graduated measuring cylinder (100 ml), homeo vials, measuring cylinder and Petri plates.

3.4 Fungus

Cultures of *R. solani* (ITC4576) and *S. rolfsii* (ITCC6856) were obtained from Indian Type Culture Collection (ITCC) Center, Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi. The cultures were maintained on PDA slants at 25 ºC and were sub cultured prior to bioassay.

3.5 Test Insect

The larvae of *S. litura* collected from the field of Indian Agricultural Research Institute (IARI), New Delhi, and identified by Dr. Chitra Srivastava, Head, Division of Entomology, IARI, New Delhi, India. The larvae reared under laboratory conditions 60±5% relative humidity and 3rd instar larvae were used for bioassay.

3.6 Methods

3.6.1 Preparation of primary emulsion of neem (PEN)

Primary emulsions were prepared using spontaneous emulsification procedure (Chang *et al.,* 2013). In brief, neem oil and surfactant (Tween-20) were first mixed together in different ratios and slowly added into aqueous phase drop wise followed by stirring (600 rpm) for 6 hours at room temperature. Different proportions of neem oil, surfactant and water (1:1:8, 0.5:1:8.5, 1:0.5:8.5, 0.5:0.5:9) were standardized.

3.6.2 Preparation of primary emulsion of citronella (PEC)

Similarly, emulsions containing various ratios of citronella oil, surfactant (Triton-X100) and distilled water (1:1:8, 0.5:1:8.5, 1:0.5:8.5, 0.5:0.5:9) were used for preparation of primary emulsion and their ratio standardized.

3.6.3 Preparation of neem nanoemulsion (NNE) with citronella oil

Standardized primary emulsion of neem (neem oil, surfactant and water (0.5:1.0:8.50) was used to prepare neem nanoemulsion (NNEs) with different concentrations of citronella oil (0.5-5.0%) by similar procedure as stated above.

3.6.4 Preparation of citronella nanoemulsion (CNE) with neem oil

Similarly, standardized primary emulsion of citronella (citronella oil, surfactant and water (0.5:1.0: 8.5) was used to prepare citronella nanoemulsion (CNE) with different concentration of neem oil (0.50-5.00%) by similar procedure mentioned above.

3.6.5 Characterization of developed formulations

3.6.5.1 Determination of particle size of nanoemulsions

The micelle size of the nanoemulsions were determined using a dynamic light scattering instrument (Zetatrac^{TM}), according to a method reported previously (Ali *et al.,* 2017), is based on Dynamic Light Scattering (DLS) which detects the fluctuation

of the scattering intensity due to the Brownian motion of particles in suspension. DLS measurements were performed at 25 ^{o}C and light scattering was detected at a fixed angle. 200 mg L^{-1} sample solution was prepared in distilled water. 5 ml of the sample solution was taken into a glass vial. Minimum quantity (50 µL) of chloroform was added to the emulsions and the vial was sonicated for 5 minutes to form a proper emulsion. Dual optical probe technology was used for particle size analysis. Optical light sources were dual solid-state laser diodes in 780 nm (near-infrared) wavelength.

3.6.5.2 Determination of morphology of nanoemulsions

The morphology of the neem nanoemulsions and citronella nanoemulsions were studied by transmission electron microscope (TEM). In this analysis, a drop of neem nanoemulsions and citronella nanomulsions were placed on carbon-coated copper grids (400 mesh size) and allowed to dry in vacuum (Baboota *et al.,* 2007), washed with distilled water and stained with a negative stain (2% uranyl acetate). The excess water was dried gently using a filter paper and then viewed under TEM at different magnification levels.

3.6.5.3 Fourier Transform Infrared Spectrophotometer (FT-IR)

The loading of citronella oil and neem oil in the nanoemulsion formulations were tested using the functional group analysis of citronella oil, neem oil, triton-X-100 and nanoemulsion employing Bruker attenuated total reflection (ATR)-Fourier Transform Infrared Spectrophotometer (ATR-FT-IR) (Model: Alpha).

3.6.5.4 Determination of nanoemulsion stability

Nanoemulsion stability was tested according to CIPAC method (MT 36.1) by using standard hard water D, prepared by method no. 18.1.4 in CIPAC hand book (CIPAC, 1970). Briefly, measuring cylinder (100 mL) was filled with standard hard water D (95 mL) at 30±1 ^{o}C. Nanoemulsion formulations (5 ml) were poured gently in measuring cylinder (100 ml) and mixed by inverting the cylinder once and after 30 sec, observation was taken. Emulsion stability on standing was tested by inverting the cylinder 10 times and allowing its content to stand undisturbed at 30±1 ^{o} C for 24 hours. The volume of any free oil, forth and cream formed at the top or at the bottom of the cylinder, after standing for 30 min, 2 hour and 24 hours were measured. Re-emulsification after standing for 24 hours was measured by inverting the cylinder 10 times and standing for 30 sec only. Final emulsion stability was measured by allowing

the cylinder to remain undisturbed for a further period of 30 min and the volume, if any, of free oil, froth, cream, or solid matter present at the end of the 30 min period was recorded.

3.6.5.5 Determination of pH of nanoemulsions

The pH of neem and citronella nanoemulsions was determined using pH meter at 20±1 ºC.

3.7. Rearing insect

A nucleus culture of *S. litura* was maintained at 25±1ºC, 60±5% relative humidity and 16:8 hour photo: scotophase on artificial diet. An artificial diet of the composition given in (Table 3.1) was used in the study. Kidney bean (*Phaseolus vulgaris*) seed procured from market were washed thoroughly and soaked overnight in water. Soaked seeds were grinded in an electric grinder thoroughly with the addition of 400 ml double distilled water. Wheat bran, wheat germ, ascorbic acid, casein, yeast powder, methyl parahydroxybenzoate, sorbic acid, cholesterol, streptomycin sulphate, formaldehyde and multivitamin (A. B. D. E. C drops) were added to the ground material and mixed thoroughly. Agar was boiled in 200 ml double distilled water with constant stirring till it attained necessary consistency and then grinded with the rest of the ingredients once again. The whole mixture was poured into plastic trays and covered with thin plastic film. After cooling, the diet was kept in refrigerator and used after 24 hours of ageing. Neonates, upon hatching from eggs, were transferred to glass jars containing fresh thoroughly washed castor leaves. Five-day old larvae were transferred to plastic boxes (30 cm long, 20 cm wide and 7 cm high) containing pieces of diet in groups of two larvae (Figures 3.1 and 3.2). Boxes were cleaned daily and larvae were fed with fresh diet. When the larvae exhibited gut purge and entered into non feeding wandering stage, they were transferred to boxes containing saw dust for pupation. Pupae were collected after four to five days and disinfected with 0.02% sodium hypochlorite. Upon emergence of adults, they were transferred to oviposition cages. Adults were fed with 20% honey solution containing vitamin C, E and streptomycin sulphate. Castor leaves with their petiole dipped in water were provided for oviposition inside the cages. All the containers used for rearing were periodically disinfected with Protasan DS® (Qualigens). This enabled to maintain a disease-free and healthy stock culture for

further experiments. Larvae for experimental purpose were reared on washed and dried castor leaves in plastic boxes. Care was taken to avoid overcrowding and strict hygiene was maintained to prevent any infection.

Table 3.1 Composition of semi-synthetic diet for *S. litura*

Content	**Quantity**
Kidney bean	65.00 g
Wheat bran	55.00 g
Wheat germ	10.00 g
Casein	3.00 g
l-ascorbic acid	4.00 g
Yeast	25.00 g
Sorbic acid	0.92 g
Methyl paraben	0.40 g
Cholesterol	0.25 g
Multivitamin (A. B. D. E. C) drops) parke-Davis India pvt. Ltd)	3 drops
Streptomycin sulphate	0.10 g
Formaldehyde	2.00 ml
Agar	10.00 g
Double distilled water	600 ml

Figure-3.1 Experimental set up to evaluate the insect growth regulatory activity against *Spodoptera litura*

Figure-3.2 3rd instar larva of *Spodoptera litura*

3.8 Bioassay evaluation against *R. solani* and *S. rolfsii*

3.8.1 Preparation of media

In brief, potato dextrose agar (39.0 g) was suspended in distilled water (1000 ml) and sterilized in an autoclave at 15 psi for half an hour prior to use. Molten potato dextrose agar medium was poured in sterilized glass petri plate and 7.80, 15.625, 31.50, 62.50, 125 and 250 ppm of neem and citronella nanoemulsions were separately added to each petri plate, mixed under aseptic conditions in a laminar flow and allowed the media to solidify. Each plate was inoculated with *R. solani* and *S. rolfsii* using 5 mm thick disc of fungus (spores and mycelium) along with control and incubated at 25±1 ºC till the fungal growth in the control petri plate was almost complete. The incubation period was observed to be 4-5 days for *R. solani* and 3-4 days for *S. rolfsii*. The antifungal activity was evaluated by measuring the relative growth of fungus in treatment vis-à-vis control in triplicates. The mycelial growth (mm) of the fungi both in treated (T) and control (C) was measured diametrically in three different directions and percent inhibition in radial growth (I) was calculated using the formula (Vincent, 1947).

$$I\ (\%) = (C-T)/C*100;$$

I= Inhibition of mycelia growth
C= Growth of fungus in control
T= Growth of fungus in treatment

The inhibition (I) was converted to corrected % inhibition (IC) by using the formula

$$IC= (I-CF)/ (100-CF)*100,$$

Where CF (Correction factor) = (90-C)/C x 100, 90 is the diameter (mm) of the dishes and C is the growth of the fungus (mm) in control.

ED_{50} (mg L^{-1}) value (Effective dose for 50% inhibition) were calculate using Probit analysis package.

3.8.2 *Bioassay against S. litura*

Insect growth inhibitory activity of neem and citronella nanoemulsions were studied against the third instar larvae of *S. litura* by leaf dip method in a chamber with a controlled temperature 25±1 ºC, 60±5% relative humidity and 16 hours photophase (Sabri *et al.,* 2016). Different parameters were used to assess the insect growth regulatory (IGR) effect of the developed nanoemulsions *viz.* larval mortality, Pupal mortality, larval weight reduction, Abnormal adult, Normal adult and Insect Growth Regulatory (IGR) activity 3rd instar larvae of *S. litura* were released on treated leaves of castor for 48 hrs; dead larvae were counted and live one were transferred to plastic vial with artificial diet for further observation. Larval mortality and larval weight reduction after 24 hrs, 48 hrs and 72 hrs were recorded; larvae were counted as dead when they were unable to move even after pressing with a brush. Larval weight reduction was recorded. Abnormal adults were with shrunken and partially stretched/wrinkled wings. These adults were unable to fly and died after some time. Insect growth regulatory activity and per cent reduction in larval weight reduction was calculated as follows.

$$\text{Larval mortality (\%)} = \frac{Number\ of\ dead\ larvae}{Number\ of\ test\ larvae} \times 100$$

$$\text{Larvae weight reduction (\%)} = \frac{Weight\ gain\ in\ control - weight\ gain\ in\ treatment}{Weight\ gain\ incontrol} \times 100$$

$$Malformation\ (\%) = \frac{Number\ of\ abnormal\ adults}{Number\ of\ test\ larvae} \times 100$$

$$Insect\ growth\ regulatory\ activity\ (\%) = 100 - Percent\ normal\ adults$$

3.9 Statistical analysis

All the data recorded were subjected to analysis of variance (ANOVA), after transformation of data in a completely randomized block design (CRB). The analyses were carried out using (SAS 9.4) software package.

4. RESULTS

4.1 Preparation of primary and nanoemulsions

Neem oil, Tween-20 and distilled water were used as ingredients for the preparation of primary oil-in-water (O/W) emulsions of neem oil. Similarly, citronella oil, Triton-X 100 and distilled water were used for the preparation of (O/W) primary emulsions of citronella oil. Studying, the different combination of ingredients, it was observed that the best ratio of oil, surfactant and distilled water for making primary emulsions of both the oils was 0.5:1.0:8.5. The increase in surfactant to neem oil or citronella oil ratio caused a decrease in droplet size. Ten neem nanoemulsions with different concentration of citronella oil and ten citronella nanoemulsions with different concentrations of neem oil were prepared and characterized (Figure-4.1).

4.2 Droplet size of neem nanoemulsions

The droplet size of various neem nanoemulsions are shown in (Table 4.1.) The average droplet size of neem nanoemulsions with different percentage of citronella oil ranged from 11.23±3.86 nm to 17.80±4.52 nm (Figure-4.2) The prepared nanoemulsions with 1.0 and 1.5% citronella oil (NNE-2 and NNE-3) showed the minimum droplet size and were found to be 11.23±2.78 and 11.23±3.86 nm, respectively.

The size of droplets of the citronella nanoemulsions are shown in (Table 4.2 and Figure-4.3.) The average droplet size of citronella nanoemulsions with different concentrations of neem oil ranged from 8.12±2.80 nm to 12.04±3.74 nm and nanoemulsion with 4.50% neem oil (CNE-9) showed the smallest size 8.12±2.80 nm (Figure-4.4). The droplet size was found to decrease with increase in surfactant concentration.

Table 4.1 Composition of neem nanoemulsions (NNEs) and their size

SL No.	Nanoemulsion composition			Code	Size of nanomicelle (nm)
	Neem oil (%)	Citronella oil (%)	Tween-20 (%)		
1	5	0.5	1	NNE-1	12.34± 3.47
2	5	1.0	1	NNE-2	11.2± 2.78
3	5	1.5	1	NNE-3	11.23± 3.86
4	5	2.0	1	NNE-4	12.69± 3.99
5	5	2.5	1	NNE-5	13.84± 4.18
6	5	3.0	1	NNE-6	11.60± 6.00
7	5	3.5	1	NNE-7	15.45± 4.11
8	5	4.0	1	NNE-8	15.30± 4.40
9	5	4.5	1	NNE-9	14.75± 4.05
10	5	5.0	1	NE-10	17.80± 4.52

Table 4.2 Composition of citronella nanoemulsions (CNEs) and their size

SL No.	Nanoemulsion composition			Code	Size of nanomicelle (nm)
	Citronella oil (%)	Neem oil (%)	Triton-X 100 (%)		
1	5	0.5	1	CNE-1	10.25 ±3.47
2	5	1.0	1	CNE-2	10.88 ±3.17
3	5	1.5	1	CNE-3	12.04 ±3.74
4	5	2.0	1	CNE-4	10.88 ±3.44
5	5	2.5	1	CNE-5	10.25 ±1.64
6	5	3.0	1	CNE-6	10.68 ±1.59
7	5	3.5	1	CNE-7	11.24 ±1.60
8	5	4.0	1	CNE-8	11.26 ±2.16
9	5	4.5	1	CNE-9	8.12 ±2.80
10	5	5.0	1	CNE-10	8.70 ±2.07

Figure-4.1 Neem nanoemulsions with different percent of citronella oil

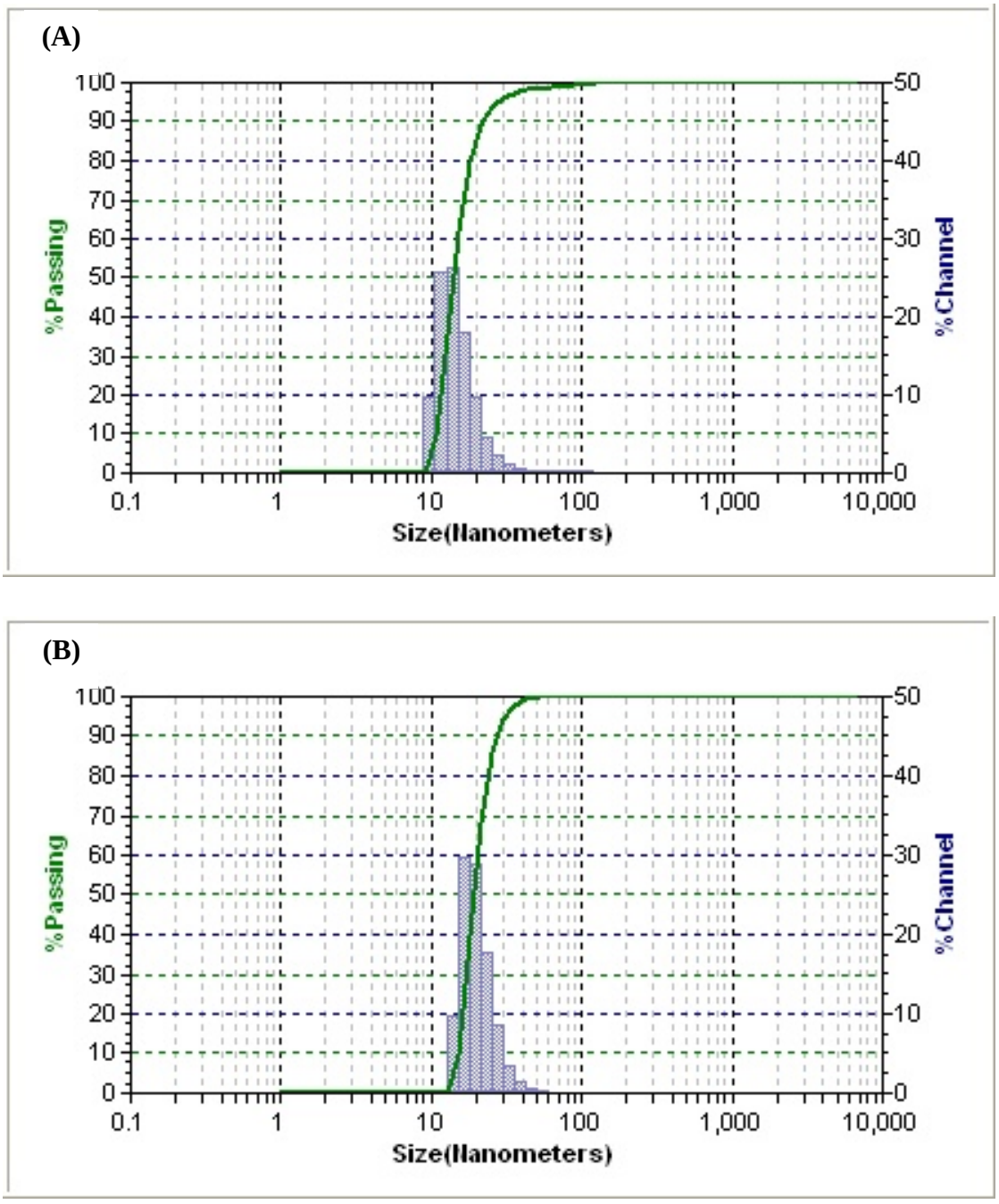


Figure-4.2 Micelle size of (A) NNE-4 (PEN+2.0% Citronella oil) and (B) NNE-10 (PEN+5.0% Citronella oil) as measured by DLS

Figure-4.3 Citronella nanoemulsions with different percent of neem oil

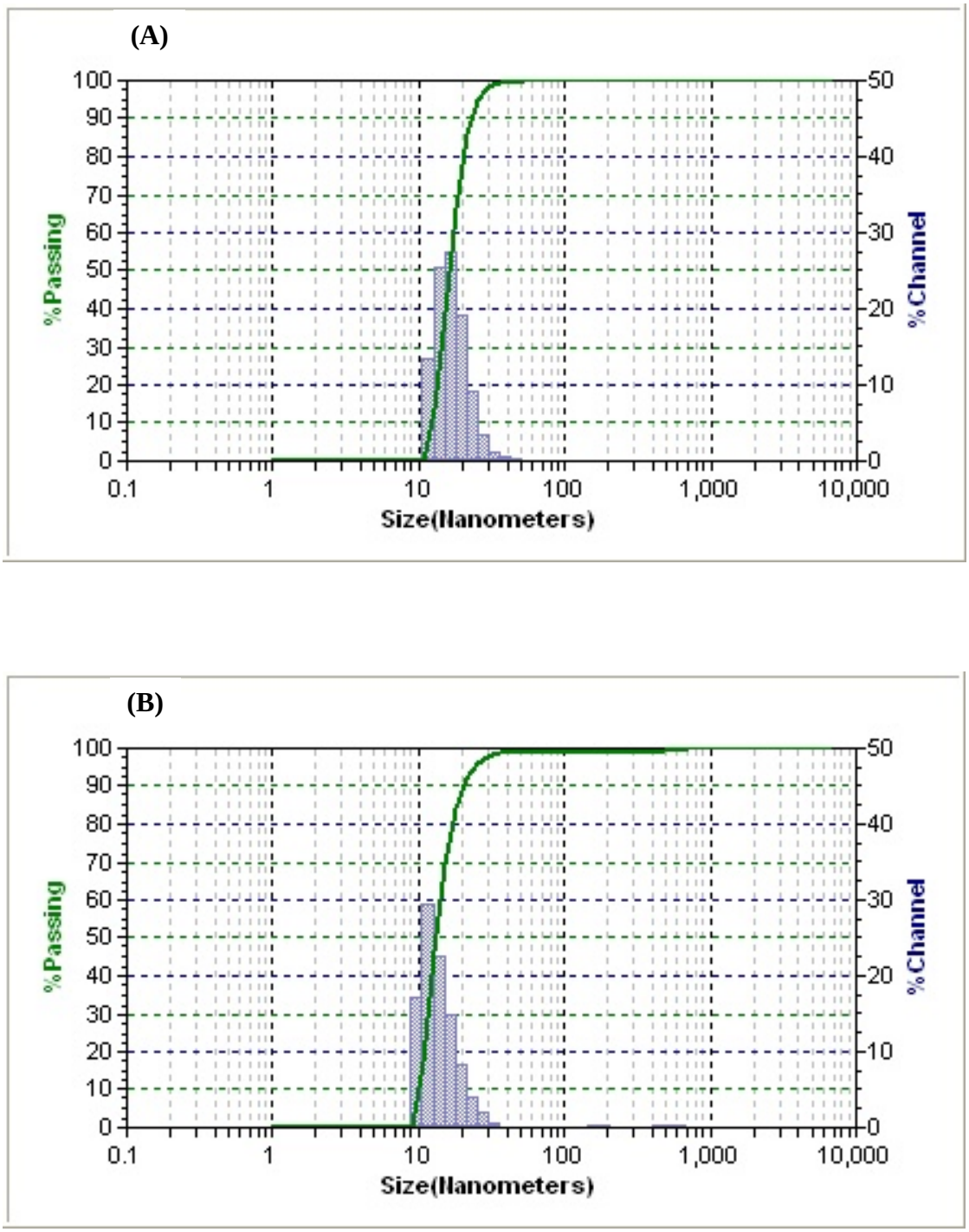


Figure-4.4 Micelle size of (A) CNE-9 (PEN+4.50% Neem oil) and (B) CNE-3 (PEN+1.50% Neem oil) as measured by DLS

4.3 Morphology of nanoemulsions

The morphology of neem and citronella oil nanoemulsions was confirmed by Transmission Electron Microscope (Figures-4.5 and 4.6). Results showed that some nanomicelle clusters were present in the nanoemulsions, which may be due to aggregation of nanomicelles formed during sample preparation, causing variation in particle size. Transmission electron microscopic study also revealed the presence of the spherical particles in nanoemulsions.

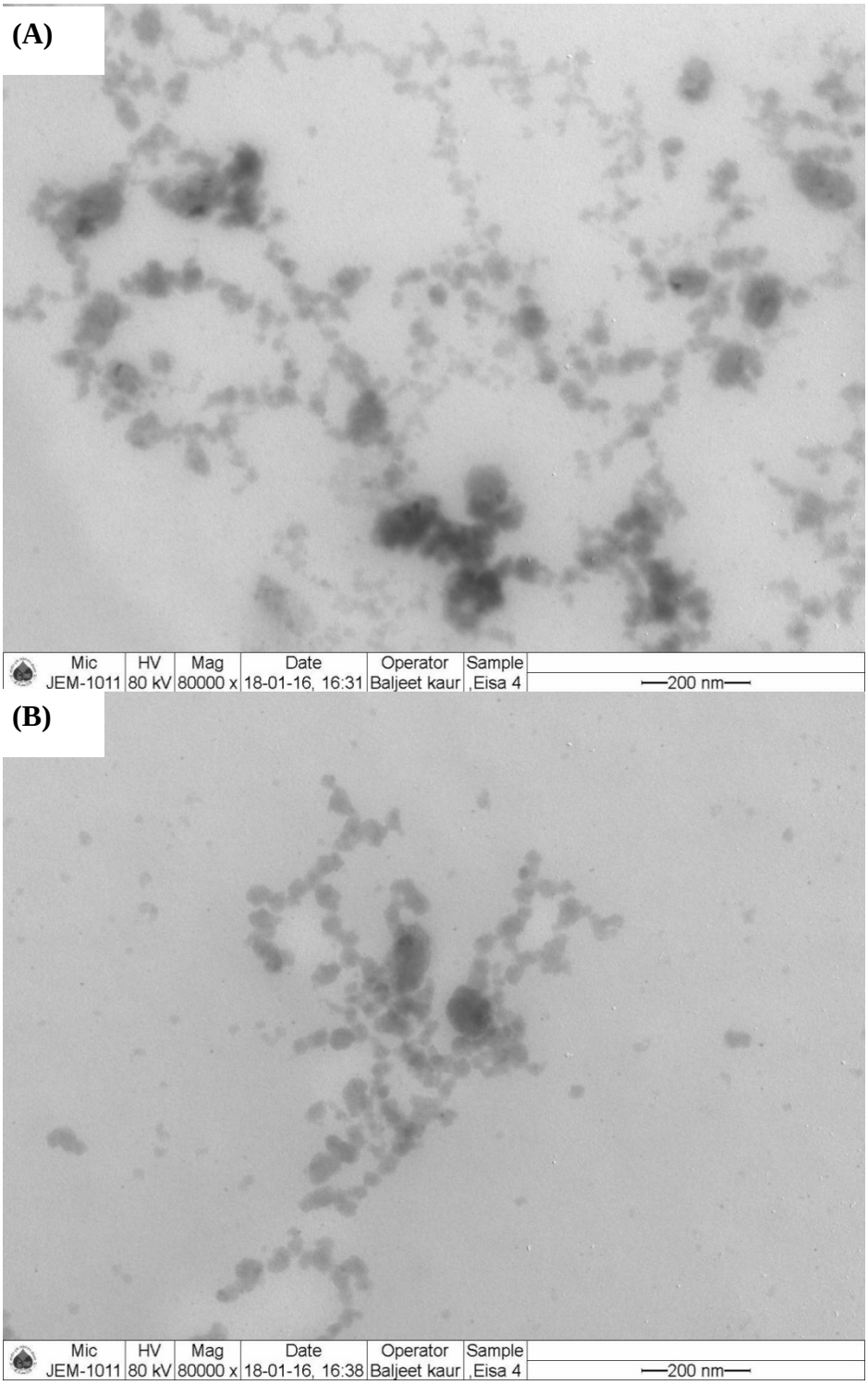


Figure-4.5. Transmission electron microscopy (TEM) image of (A) NNE-4 (PEN+2.0% Citronella oil) and (B) NNE-10 (PEN+5.0% Citronella oil)

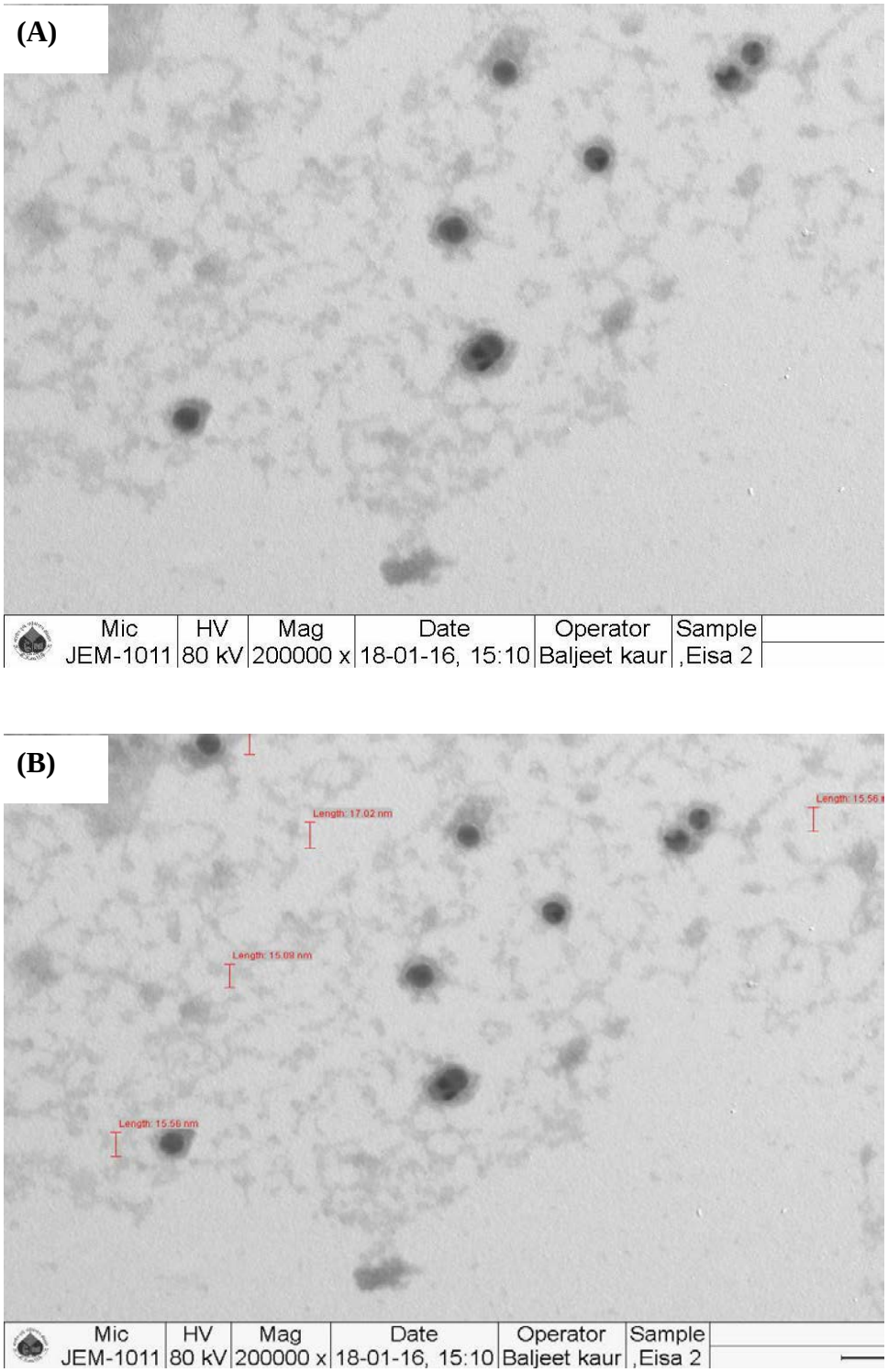


Figure-4.6 Transmission electron microscopy (TEM) image of (A) CNE-4 (PEC+2.0% Neem oil) and (B) CNE-10 (PEC+5.0% Neem oil)

4.4 Fourier Transform Infrared Spectrophotometer (FT-IR)

The FT-IR spectra of citronella oil, neem oil, Triton-X-100 and nanoemulsions are shown in (Figure-4.7 and Table 4.3.) Citronella oil showed major bands at 3365, 2925, 1724 and 1669 cm^{-1} corresponding to OH stretching, methylene C-H asymmetric stretching, carbonyl stretching and olefinic unsaturation stretching, respectively. The characteristic bands of neem oil were observed at 2922, 2852, 1744, 1464 and 1160 cm^{-1} corresponding to OH stretching, aromatic/vinyl C-H stretching, aliphatic C-H stretching, carbonyl stretching and ether stretching, respectively. The developed nanoemulsion showed characteristics bands of both citronella oil and neem oil. The characteristic bands of Triton-x-100 were not clear in the FT-IR spectra of nanoemulsion due to similarity of functional groups with oils.

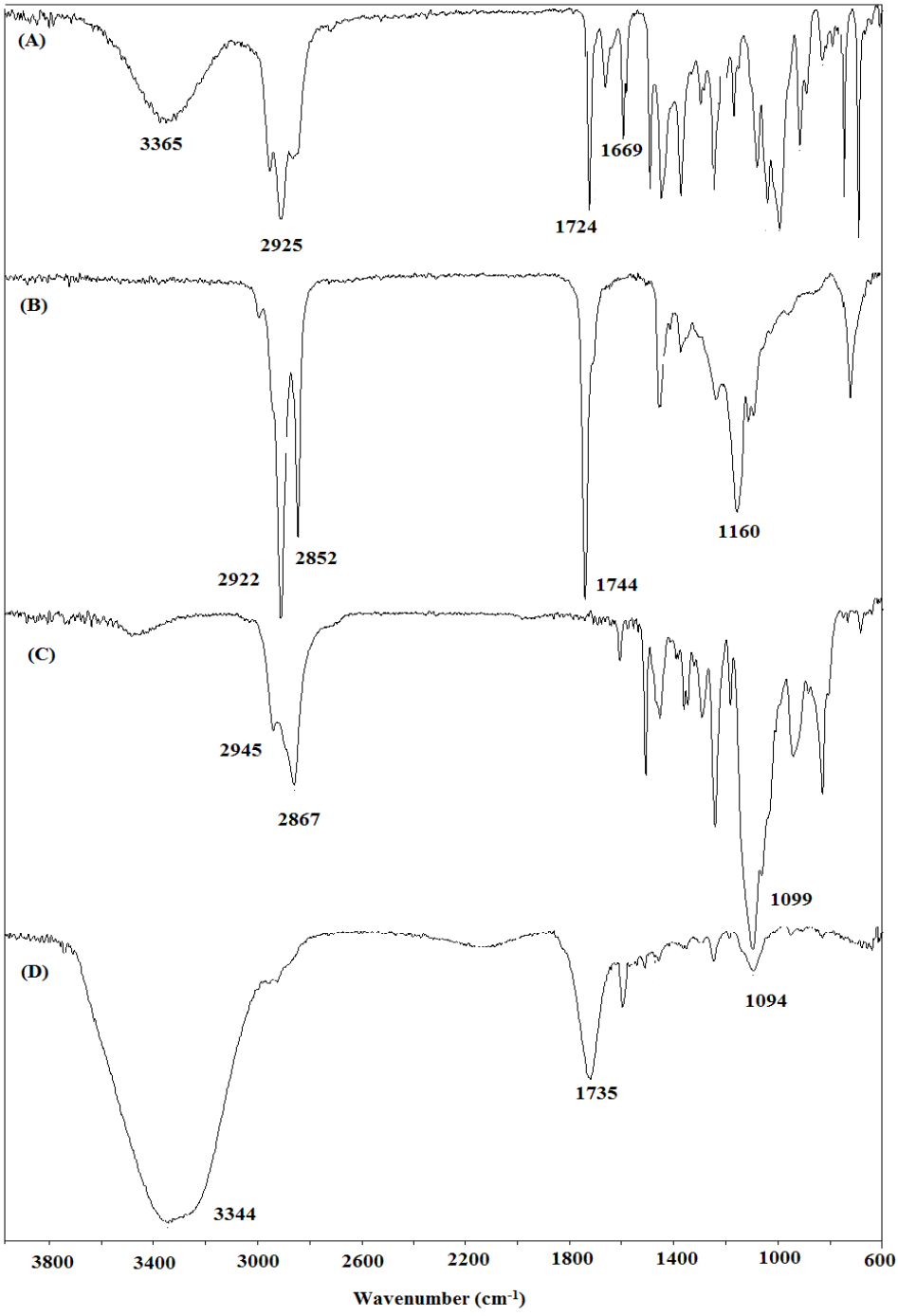


Figure-4.7 FT-IR spectra of citronella oil (A), neem oil (B), Triton-X-100 (C) and representative nanoemulsion (CNE-10) (D)

Table 4.3 FT-IR data showing major functional groups in citronella oil, neem oil, Triton-X-100 and nanoemulsion

	Wave number (cm^{-1})	Functional group assigned
Citronella Oil	3365	O-H stretching
	2925	Methylene C–H asymmetric stretching
	1724	C=O stretching
	1669 Stretching	Olefinic unsaturation C=C
Neem Oil	2922	Alkenyl C–H stretching
	2852	Alkyl C-H stretching
	1744	C=O stretching
	1464	Alkyl C-H bending
	1160	C-O stretching
Triton-X-100	2945	Aromatic C–H stretching
	2867	Alkyl C-H stretching
	1099	C-O stretching
Nanoemulsion	3344	O-H stretching
	1735	C=O stretching
	1094	C-O stretching

4.5 Emulsion stability

No foaming or separation of oily matter was observed in any of the developed NNEs. On dilution with standard hard water D, NNEs formed stable, nearly transparent emulsion. The emulsion stability on standing was checked and found that no free oil, frothing or any cream was formed in the test cylinders. Again on re-emulsification, no abnormality and separation of cream, froth and oil was observed. The image of the emulsion stability test is shown in (Table 4.4 and Figure-4.8.)

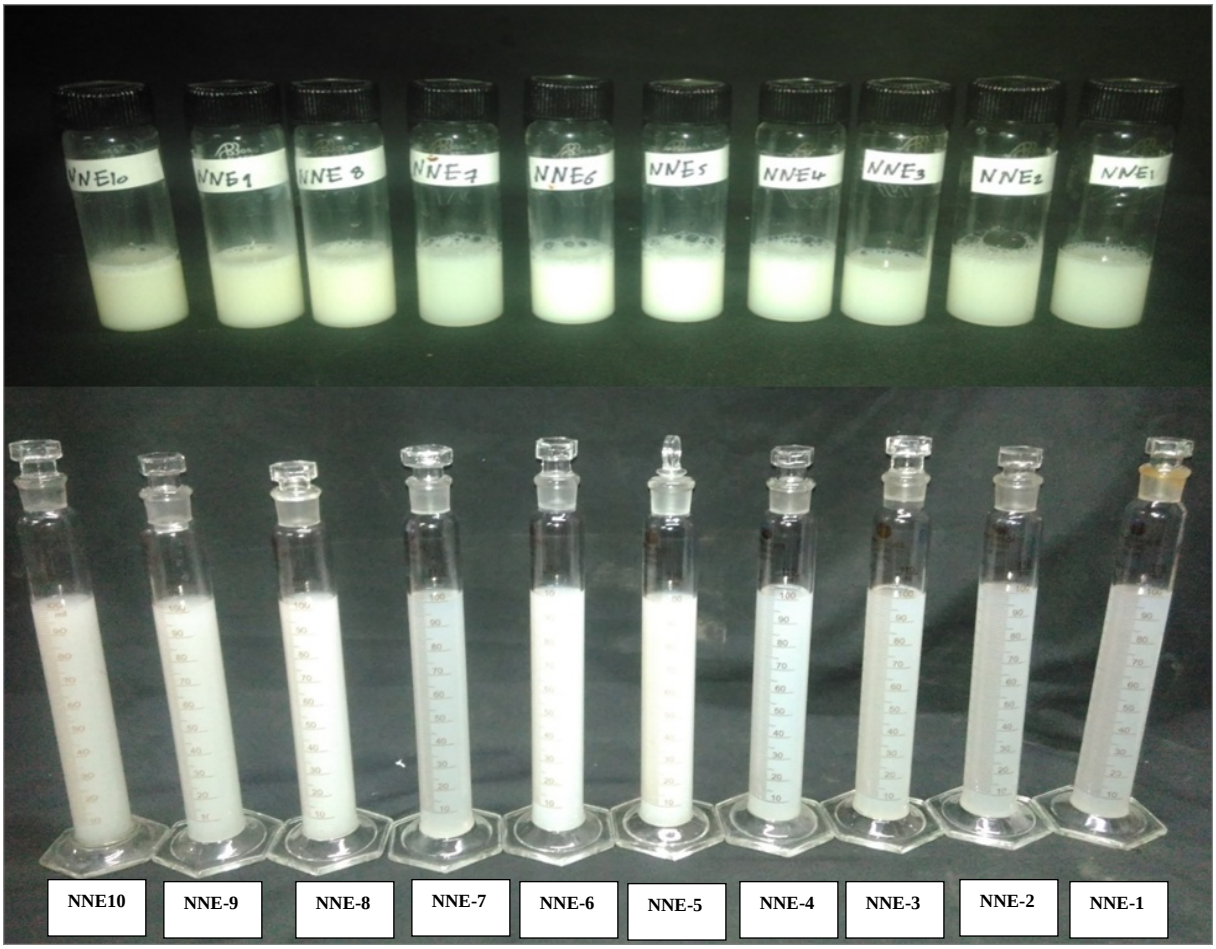


Figure-4.8 Images of neem nanoemulsions (NNEs) and their stability test after 24 hrs.

Table 4.4 Emulsion stability and re-emulsification of neem nanoemulsions

Formulations	Initial emulsification		Emulsion stability on standing						Re-emulsification after standing for 24 hrs		Final emulsion stability for 24hrs	
	30 Sec		30 min		2 hrs		24 hrs		30 sec		30 min	
	Froth (ml)	Free oil (ml)	Froth (ml)	Free oil (ml)	Froth (ml)	Free oil (ml)	Froth (ml)	Free oil (ml)	Froth (ml)	Free oil (ml)	Froth (ml)	Free oil (ml)
NNE-1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NNE-2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NNE-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
NNE-4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
NNE-5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NNE-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NNE-7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
NNE-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NNE-9	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
NNE-10	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0

Likewise, the developed citronella nanoemulsions (CNEs) showed high stability as no foaming and separation of oily matter was observed. On dilution with standard hard water D, CNEs formed stable nearly transparent emulsion. The emulsion stability on standing was checked and found that no free oil, frothing or any cream was formed in the test cylinders. Again on re-emulsification, no abnormality and separation of cream, froth and oil was observed (Table 4.5 and Figure-4.9).

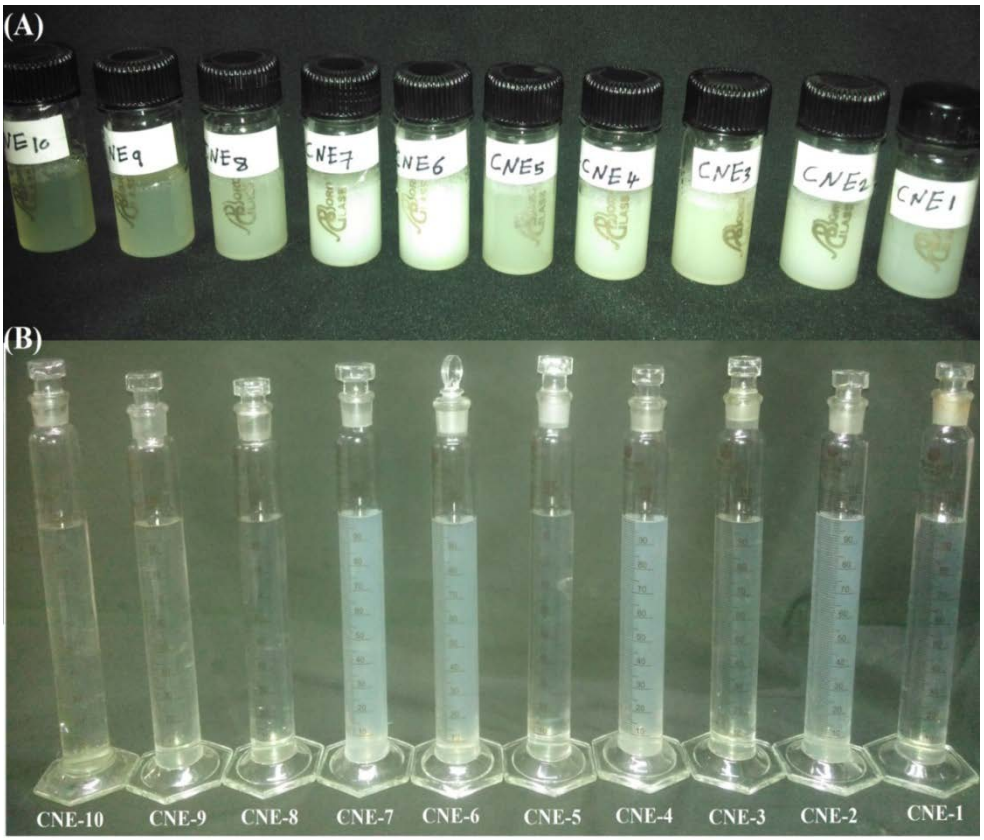


Figure-4.9 Images of citronella nanoemulsions (CNEs) and their stability test after 24 hrs.

Table 4.5 Emulsion stability and re-emulsification of citronella nanoemulsions

Formulations	Initial emulsification		Emulsion stability on standing						Re-emulsification after standing for 24 hrs		Final emulsion stability for 24hrs	
	30 Sec		30 min		2 hrs		24 hrs		30 sec		30 min	
	Froth (ml)	Free oil (ml)	Froth (ml)	Free oil (ml)	Froth (ml)	Free oil (ml)	Froth (ml)	Free oil (ml)	Froth (ml)	Free oil (ml)	Froth (ml)	Free oil (ml)
CNE-1	0.60	0.00	0.40	0.00	0.30	0.00	0.00	0.00	0.60	0.00	0.40	0.00
CNE-2	0.40	0.00	0.40	0.00	0.30	0.00	0.00	0.00	0.70	0.00	0.20	0.00
CNE-3	0.20	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00
CNE-4	0.40	0.00	0.30	0.00	0.20	0.00	0.00	0.00	0.70	0.00	0.30	0.00
CNE-5	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00
CNE-6	0.50	0.00	0.60	0.00	0.40	0.00	0.00	0.00	0.60	0.00	0.20	0.00
CNE-7	0.60	0.00	0.40	0.00	0.20	0.00	0.00	0.00	0.60	0.00	0.40	0.00
CNE-8	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00
CNE-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00
CNE-10	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00

4.6 pH of nanoemulsions

The pH of neem and citronella oil nanoemulsions measured is given in (Table 4.6.) The pH values of neem nanoemulsions ranged from 3.53-4.51 and that of citronella nanoemulsions from 2.30 to 5.85. No relation was found for the pH change with increasing concentrations of plant oils in nanoemulsions.

Table 4.6 pH values of neem nanoemulsions and citronella nanoemulsions

Sr.No.	Citronella nanoemulsion (CNE)	pH	Sr.No.	Neem nanoemulsion (NNE)	pH
1.	CNE-1	2.39	1.	NNE-1	4.51
2.	CNE-2	2.51	2.	NNE-2	3.53
3.	CNE-3	5.38	3.	NNE-3	3.66
4.	CNE-4	5.83	4.	NNE-4	3.79
5.	CNE-5	5.85	5.	NNE-5	3.96
6.	CNE-6	5.38	6.	NNE-6	4.51
7.	CNE-7	4.72	7.	NNE-7	3.71
8.	CNE-8	5.26	8.	NNE-8	3.66
9.	CNE-9	4.80	9.	NNE-9	3.75
10.	CNE-10	4.65	10.	NNE-10	4.43

4.7 Antifungal activity of neem nanoemulsions against *Rhizoctonia solani* and *Sclerotium rolfsii*

Different concentrations *viz.*, 7.80, 15.625, 31.25, 62.50, 125 and 250 ppm of neem nanoemulsions were screened for their antifungal activity against *R. solani* and *S. rolfsii* by poisoned food technique (Sharma and Tripathi, 2008) (Table 4.7 and Figures-4.10, 4.11, 4.12 and 4.13) ED_{50} values were found to vary from 13.69 to 109.71 mg L^{-1} for *R. solani* and 14.71 to 85.97 mg L^{-1} for *S. rolfsii* (Figure-4.14).

Table 4.7 Antifungal activity of neem nanoemulsions against *R. solani* and *S. rolfsii*

Nanoemulsions	Conc. (ppm)	*R. solani*		*S. rolfsii*	
		Inhibition (%)	ED_{50} (ppm)	Inhibition (%)	ED_{50} (ppm)
NNE-1	250.00	61.74^{efgh}	109.71^{b}	61.07^{fghijk}	85.97^{b}
	125.00	50.26^{ijklm}		$55.59^{ijklmnopq}$	
	62.50	$40.81^{mnopqrs}$		41.19^{stu}	
	31.25	35.44^{qrstu}		41.15^{stu}	
	15.63	33.59^{rstuv}		40.85^{stuv}	
	7.80	18.89^{yz}		17.93^{z}	
NNE-2	250.00	64.11^{defg}	98.37^{bc}	63.26^{efghij}	70.32^{bc}
	125.00	$49.19^{ijklmno}$		$60.26^{fghijkl}$	
	62.50	$42.63^{klmnopqrs}$		41.48^{stu}	
	31.25	37.52^{pqrst}		42.33^{rstu}	
	15.63	35.07^{qrstu}		41.74^{stu}	
	7.80	21.22^{xy}		20.85^{yz}	
NNE-3	250.00	65.22^{cdef}	82.01^{bcd}	64.44^{defghi}	56.67^{cd}
	125.00	51.81^{hijkl}		63.26^{efghij}	
	62.50	$44.44^{jklmnopq}$		44.89^{qrst}	
	31.25	39.33^{opqrs}		46.81^{opqrst}	
	15.63	37.11^{pqrst}		42.30^{rstu}	
	7.80	22.22^{xy}		22.07^{xyz}	
NNE-4	250.00	66.11^{bcdef}	63.62^{cde}	65.78^{defghi}	47.62^{de}
	125.00	55.56^{fghi}		$65.85^{cdefghi}$	
	62.50	$46.70^{jklmnop}$		47.07^{opqrs}	
	31.25	40.07^{nopqrs}		48.33^{nopqrs}	
	15.63	38.48^{pqrst}		45.07^{pqrst}	
	7.80	29.15^{tuvwx}		23.70^{xyz}	
NNE-5	250.00	69.48^{abcde}	54.70^{de}	$70.85^{abcdefg}$	38.13^{ef}
	125.00	61.41^{efgh}		$68.78^{bcdefgh}$	
	62.50	49.59^{ijklmn}		48.48^{nopqrs}	
	31.25	$41.67^{mnopqrs}$		$51.30^{klmnopqrs}$	
	15.63	39.11^{pqrst}		47.11^{opqrs}	
	7.80	23.00^{wxy}		24.22^{xyz}	
NNE-6	250.00	72.41^{abcd}	38.56^{ef}	72.96^{abcde}	36.05^{ef}
	125.00	64.52^{def}		$68.15^{bcdefgh}$	
	62.50	51.85^{hijkl}		$49.37^{mnopqrs}$	
	31.25	$46.96^{jklmnop}$		$53.33^{jklmnopq}$	
	15.63	$40.22^{mnopqrs}$		47.41^{opqrs}	
	7.80	32.89^{stuvw}		24.44^{xyz}	

NNE-7	250.00	74.67^{abc}	24.69^{ef}	76.96^{ab}	27.77^{f}
	125.00	66.26^{bcdef}		70.89^{abcdef}	^{g}
	62.50	53.70^{ghij}		58.48^{hijklmn}	
	31.25	49.48^{ijklmn}		55.93^{ijklmn}	
	15.63	41.56^{mnopqrs}		49.81^{lmnopqrs}	
	7.80	32.89^{stuvw}		24.74^{xyz}	
NNE-8	250.00	75.04^{Abc}	27.99^{ef}	78.30^{ab}	22.79^{f}
	125.00	70.33^{abcde}		73.11^{abcde}	^{g}
	62.50	57.67^{fghi}		60.00^{ghijklm}	
	31.25	52.67^{hijk}		60.67^{fghijkl}	
	15.63	42.41^{lmnopqrs}		52.93^{jklmonpqr}	
	7.80	34.11^{rstuv}		26.56^{WXYZ}	
NNE-9	250.00	76.15^{ab}	23.91^{ef}	78.74^{ab}	18.51^{g}
	125.00	72.37^{abcd}		74.41^{abcd}	
	62.50	61.74^{efgh}		62.96^{efghij}	
	31.25	53.56^{ghij}		61.56^{fghijk}	
	15.63	43.30^{klmnopqr}		56.26^{ijklmo}	
	7.80	36.04^{qrstu}		29.89^{wxy}	
NNE-10	250.00	77.15^{a}	13.67^{f}	80.63^{A}	14.71^{g}
	125.00	74.96^{abc}		76.67^{abc}	
	62.50	66.07^{cdef}		68.30^{bcdefgh}	
	31.25	57.26^{fghi}		65.59^{defghi}	
	15.63	46.11^{jklmnop}		61.48^{fghijk}	
	7.80	37.07^{pqrst}		30.07^{vwxy}	
Neem oil	250.00	44.79^{jklmnopq}	532.04^{a}	41.24^{stu}	871.5
	125.00	26.21^{uvwxy}		36.08^{tuvw}	0^{a}
	62.50	24.64^{vwxy}		32.06^{uvwx}	
	31.25	10.97^{z}		26.57^{wxyz}	
	15.63	10.21^{z}		26.49^{wxyz}	
	7.80	10.50^{z}		16.09^{z}	
p-Value		<.0001	<.0001	<.0001	<.0001
CV (%)		5.60	11.49	5.61	4.09
SE(d)		2.146	9.116	2.322	3.919
Tukey HSD at 1%		10.063	39.902	10.885	17.155

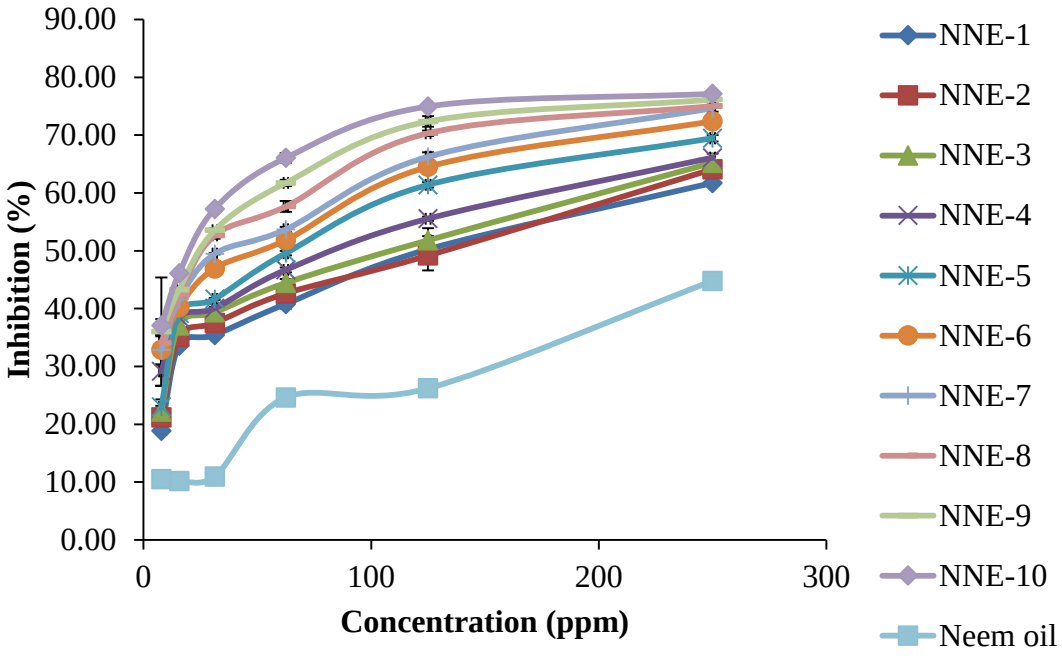


Figure-4.10 Growth inhibitory effect (%) of neem nanoemulsions against *R. solani*

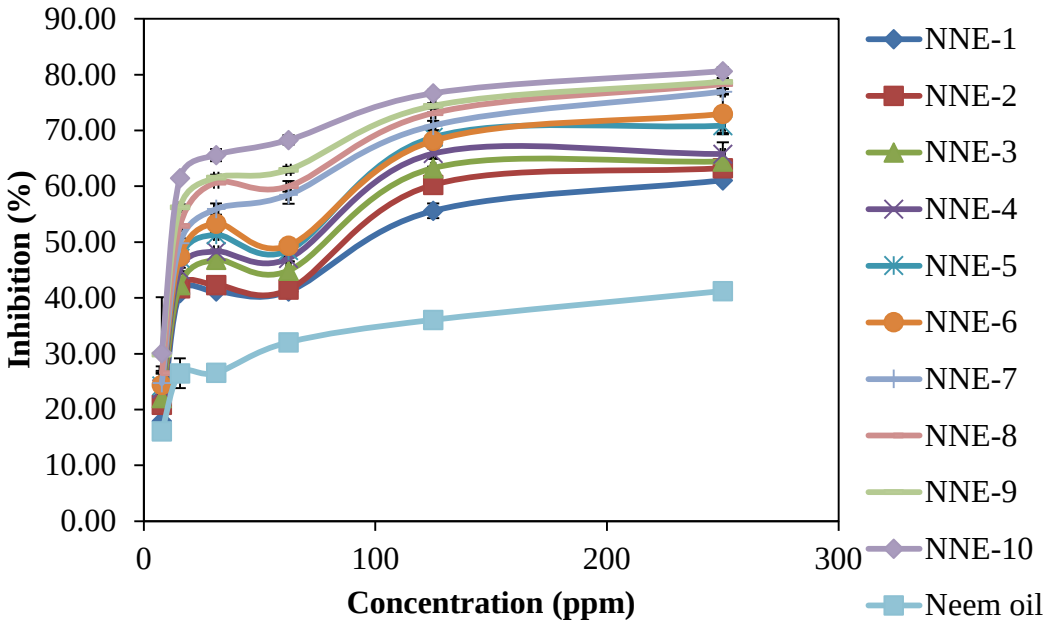
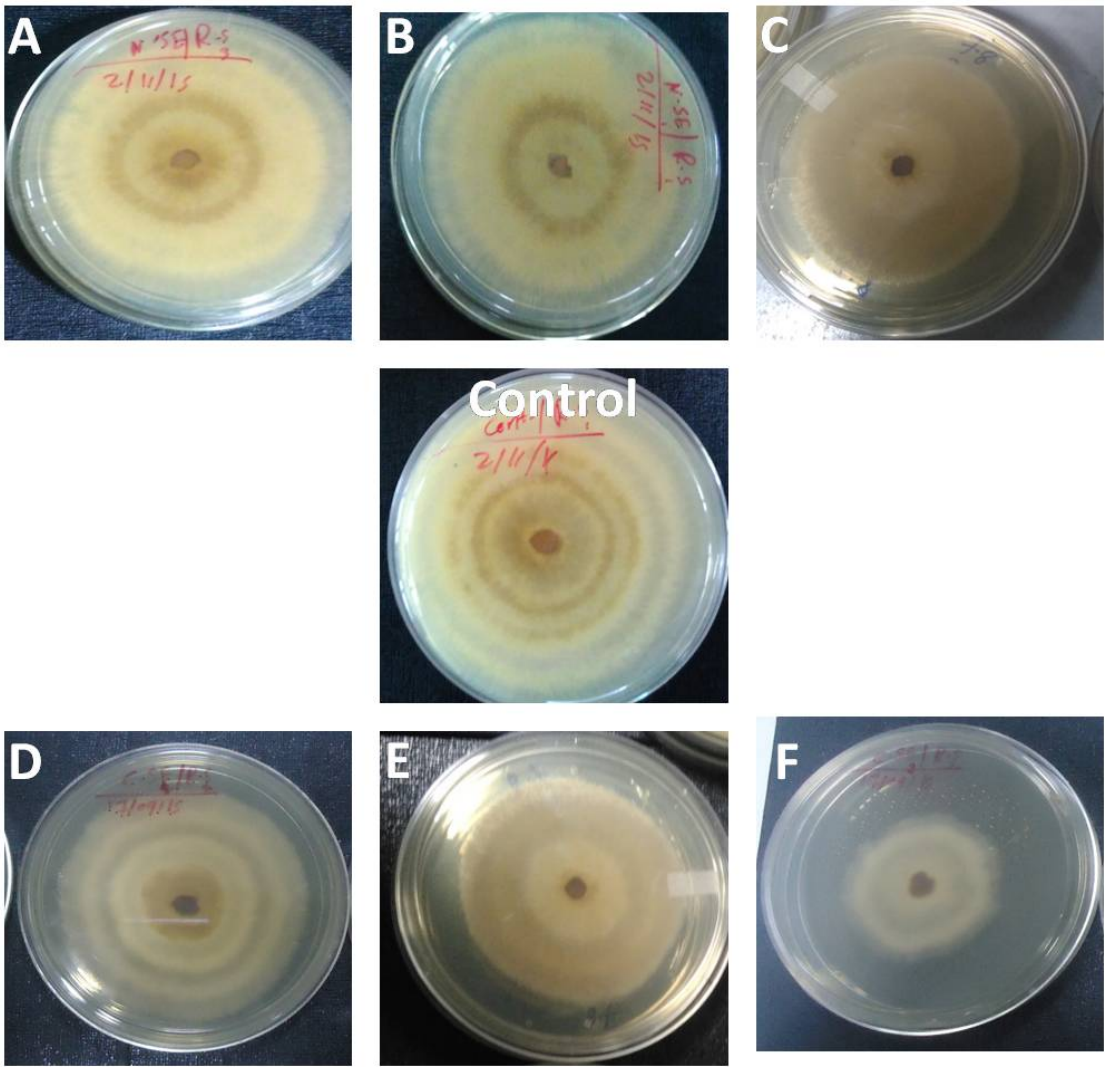
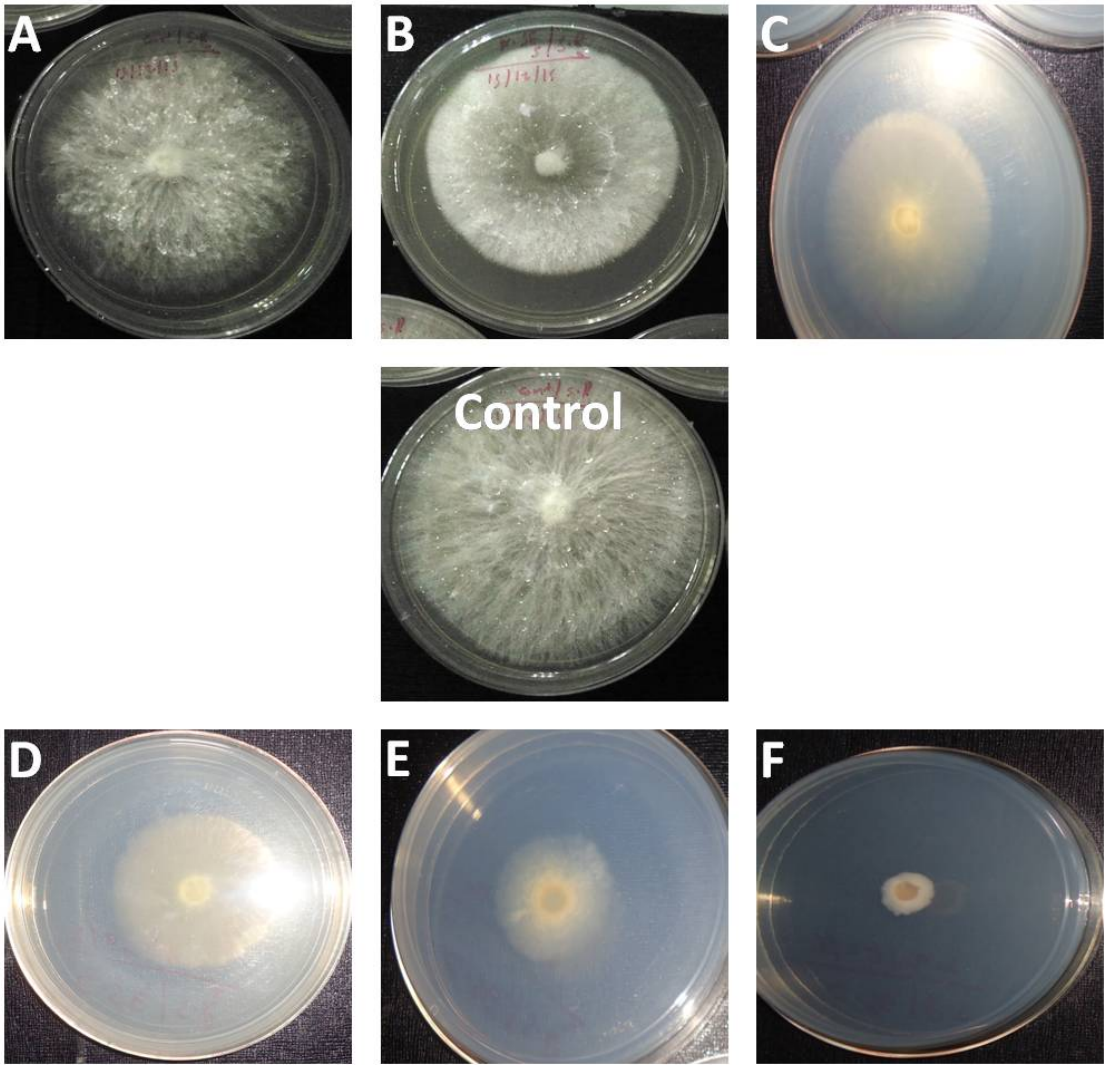


Figure-4.11 Growth inhibitory effect (%) of neem nanoemulsions against *S. rolfsii*



A=7.80 ppm, B=15.63 ppm, C=31.25 ppm D= 62.50 ppm, E=125.00 ppm, F=250.00

Figure-4.12 Fungicidal activity of neem nanoemulsion (NNE-5) against *R. solani*



A=7.80 ppm, B=15.63 ppm, C=31.25 ppm, D= 62.50 ppm, E=125.00 ppm, F=250.00

Figure-4.13 Fungicidal activity of neem nanoemulsion (NNE-5) against *S. rolfsii*

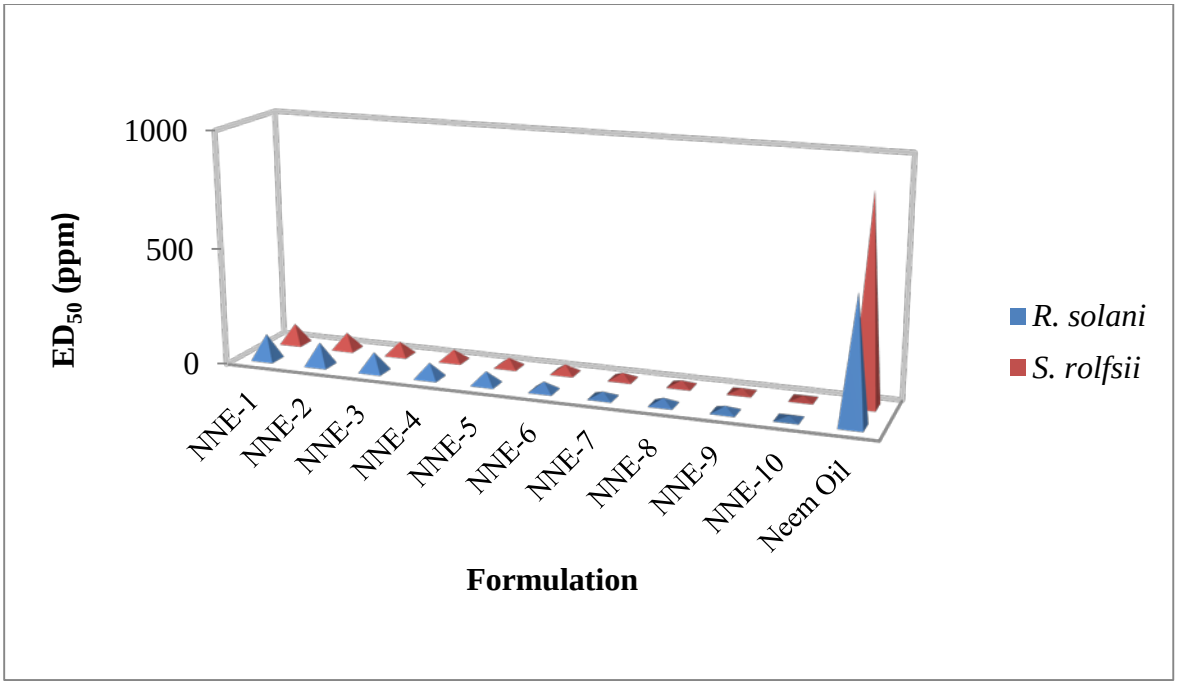


Figure-4.14 ED_{50} of neem nanoemulsions

4.8 Antifungal activity of citronella nanoemulsions against *R. solani* and *S. rolfsii*

Similarly, different concentrations (7.80, 15.625, 31.25, 62.50, 125 and 250 ppm) of citronella nanoemulsions were evaluated for their antifungal activity against *R. solani* and *S. rolfsii* by the poisoned food technique. The ED_{50} values of different nanoemulsions are given in (Table 4.8 and Figures-4.15, 4.16, 4.17 and 4.18). The results showed that the ED_{50} of citronella nanoemulusions (CNEs) against *R. solani* and *S. rolfsii* decrease with increase in the amount of neem oil (0.50 to 5.0%) Figure-4.19.

Table 4.8 Antifungal activity of citronella nanoemulsions against *R. solani* and *S. rolfsii*

Nanoemulsions	Conc. (ppm)	*R. solani*		*S. rolfsii*	
		Inhibition (%)	ED_{50} (ppm)	Inhibition (%)	ED_{50} (ppm)
CNE-1	250.00	54.19 fgh	311.0 4ab	53.22 efghi	405.75b
	125.00	37.04 rstuvw		36.30 rstu	
	62.50	35.07 uvwx		34.63 stuv	
	31.25	33.15 wxy		44.44 jklmnopqr	
	15.63	32.81 wxy		44.40 jklmnopqr	
	7.80	22.96 z		22.26 xy	
CNE-2	250.00	56.37 efg	228.62bc	56.67 cdefgh	348.66b
	125.00	40.00 nopqrstu		35.56 rstuv	
	62.50	39.96 nopqrstu		35.37 rstuv	
	31.25	35.07 uvwx		44.44 jklmnopqr	
	15.63	34.89 vwx		44.40 jklmnopqr	
	7.80	29.04 y		26.67 vwx	
CNE-3	250.00	57.52 def	302.63ab	59.63 bcde	110.62c
	125.00	40.56 nopqrs		40.74 nopqrs	
	62.50	40.00 nopqrstu		48.52 hijklmnop	
	31.25	37.26 qrstuvw		45.56 ijklmnopq	
	15.63	36.81 stuvw		40.00 pqrst	
	7.80	29.41 y		27.26 uvwx	
CNE-4	250.00	60.63 cde	166.04bc	62.74 abcd	9.98cd
	125.00	40.15 nopqrst		41.85 klmnopqrs	
	62.50	40.19 nopqrst		49.89 fghijklmn	
	31.25	38.56 pqrstuv		50.26 fghijklm	
	15.63	38.81 pqrstuv		40.60 opqrst	
	7.80	31.00 xy		31.48 tuvw	
CNE-5	250.00	62.48 cd	140.75bc	64.41 abc	59.89cd
	125.00	40.96 nopqrs		47.52 hijklmnop	
	62.50	40.22 nopqrs		50.19 fghijklm	
	31.25	39.93 nopqrstu		50.48 efghijkl	
	15.63	40.30 nopqrs		40.70 opqrst	
	7.80	32.37 wxy		33.70 stuvw	
CNE-6	250.00	63.41 bc	90.69c	65.44 abc	51.90cd
	125.00	47.56 jklm		49.33 ghijklmno	
	62.50	44.44 klmno		50.89 efghijk	
	31.25	40.33 nopqrs		50.59 efghijk	

	15.63	40.78 nopqrs		40.90 nopqrs	
	7.80	34.81 vwx		37.04 qrst	
CNE-7	250.00	64.74 bc	60.60c	68.00 ab	40.89cd
	125.00	49.44 ghi		51.85 efghi	
	62.50	47.22 ijkl		54.85 defgh	
	31.25	44.81 klmn		50.70 efghijk	
	15.63	41.96 mnopqr		41.30 mnopqrs	
	7.80	35.19 tuvwx		37.15 qrst	
CNE-8	250.00	65.30 bc	37.97c	69.41 a	35.56cd
	125.00	62.37 cd		56.67 cdefgh	
	62.50	50.93 hij		58.63 cdef	
	31.25	47.44 ijkl		50.93 efghijk	
	15.63	42.15 mnopq		41.30 lmnopqrs	
	7.80	38.89 pqrstuv		39.74 pqrst	
CNE-9	250.00	68.37 ab	29.38c	69.70 A	26.93d
	125.00	62.63 c		59.63 bcde	
	62.50	60.81 cde		62.56 abcd	
	31.25	48.63 ijk		1.11 efghij	
	15.63	41.59 nopqrs		41.90 klmnopqrs	
	7.80	39.63 opqrstuv		40.85 nopqrs	
CNE-10	250.00	70.81 a	25.64c	71.26 a	20.88d
	125.00	62.96 c		62.74 abcd	
	62.50	61.81 cd		62.67 abcd	
	31.25	51.52 ghij		58.52 cdefg	
	15.63	41.81 nopqrs		42.00 klmnopqrs	
	7.80	40.81 nopqrs		41.81 klmnopqrs	
Citronella oil	250.00	43.33 lmnop	452.90a	41.21 mnopqrs	747.89a
	125.00	28.89 y		25.36 wx	
	62.50	17.78a		18.46 xyz	
	31.25	11.67b		15.04 yza	
	15.63	8.33c		10.00 za	
	7.80	4.44c		7.22 a	
p-Value		< 0.0001	< 0.0001	< 0.0001	< 0.0001
CV (%)		3.06	35.09	5.28	12.02
SE(d)		1.072	48.093	1.962	17.203
Tukey HSD at 1%		5.0248	210.51	9.1973	75.301

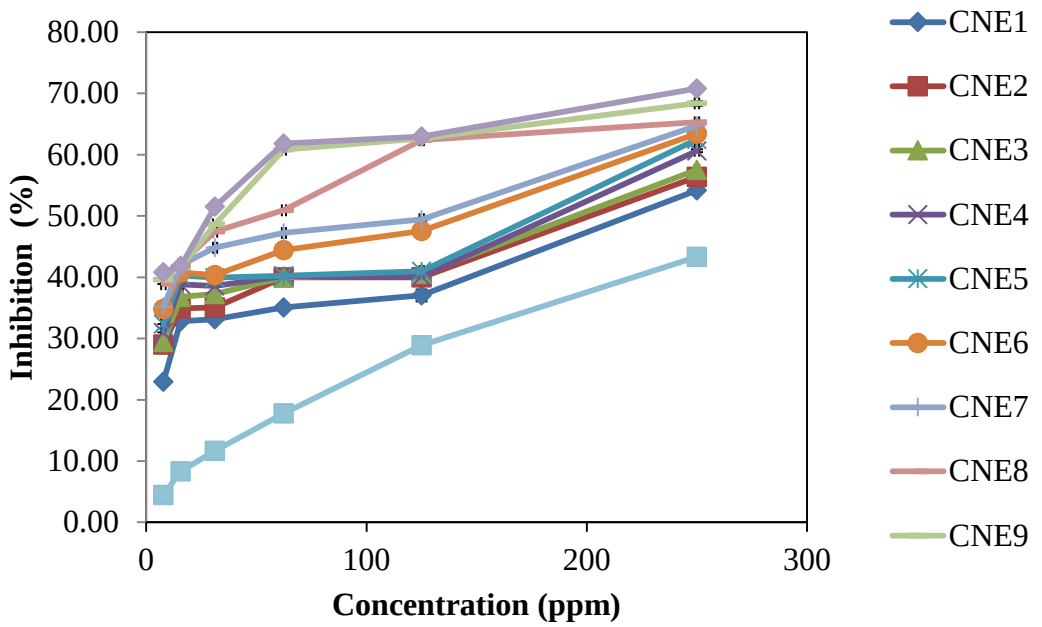


Figure-4.15 Growth inhibitory effect (%) of citronella nanoemulsions against *R. solani*

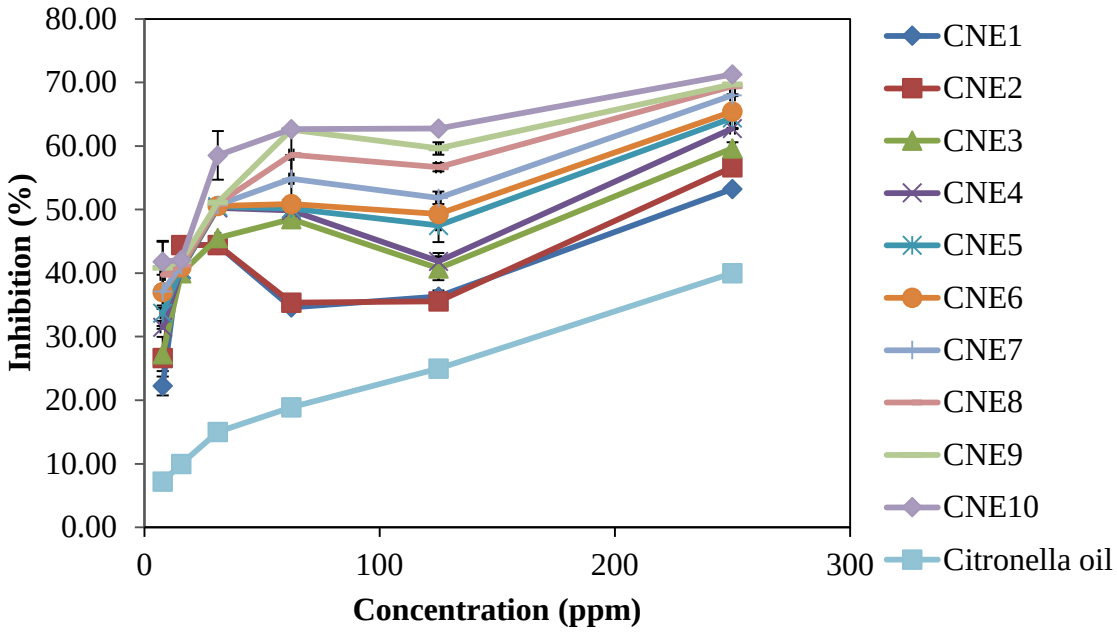
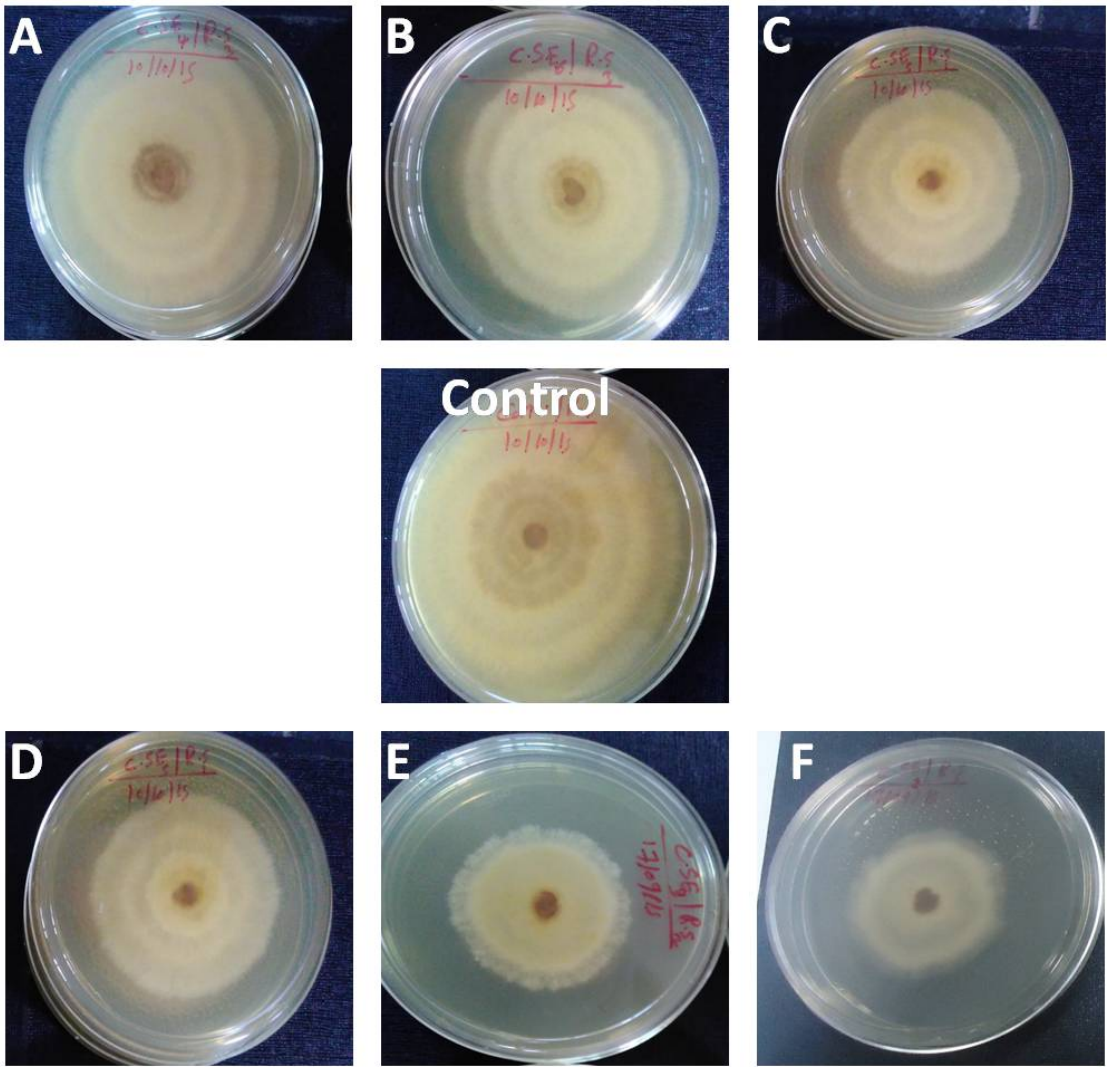
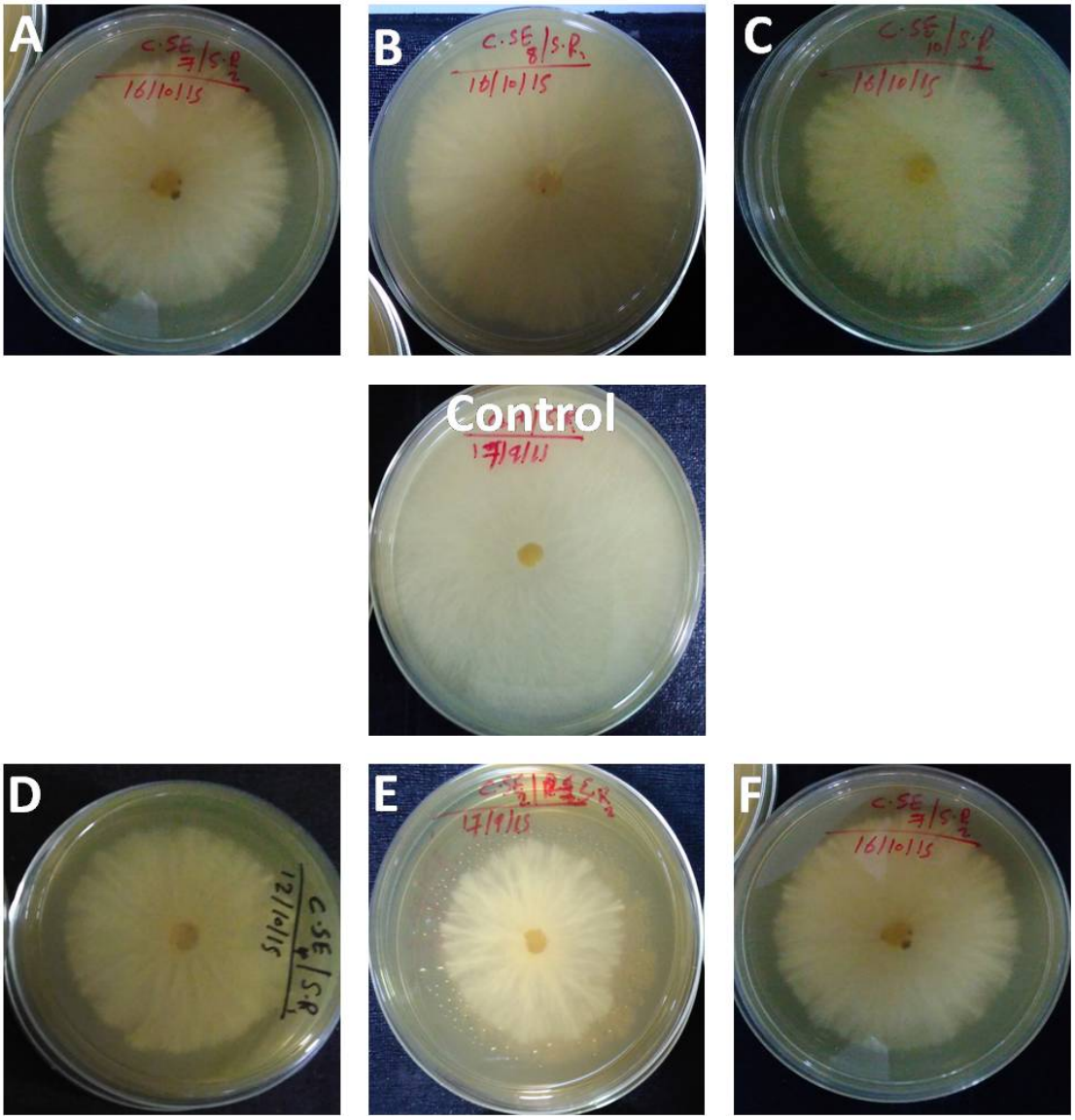


Figure-4.16 Growth inhibitory effect (%) of citronella nanoemulsions against *S. rolfsii*



A=7.80 ppm, B=15.63 ppm, C=31.25 ppm, D= 62.50 ppm, E=125.00 ppm, F=250.00

Figure-4.17 Fungicidal activity of citronella nanoemulsion (CNE-5) against *R. solani*



A=7.80 ppm, B=15.63 ppm, C=31.25 ppm, D= 62.50 ppm, E=125.00 ppm, F=250.00

Figure-4.18 Fungicidal activity of citronella nanoemulsion (CNE-5) against *S. rolfsii*

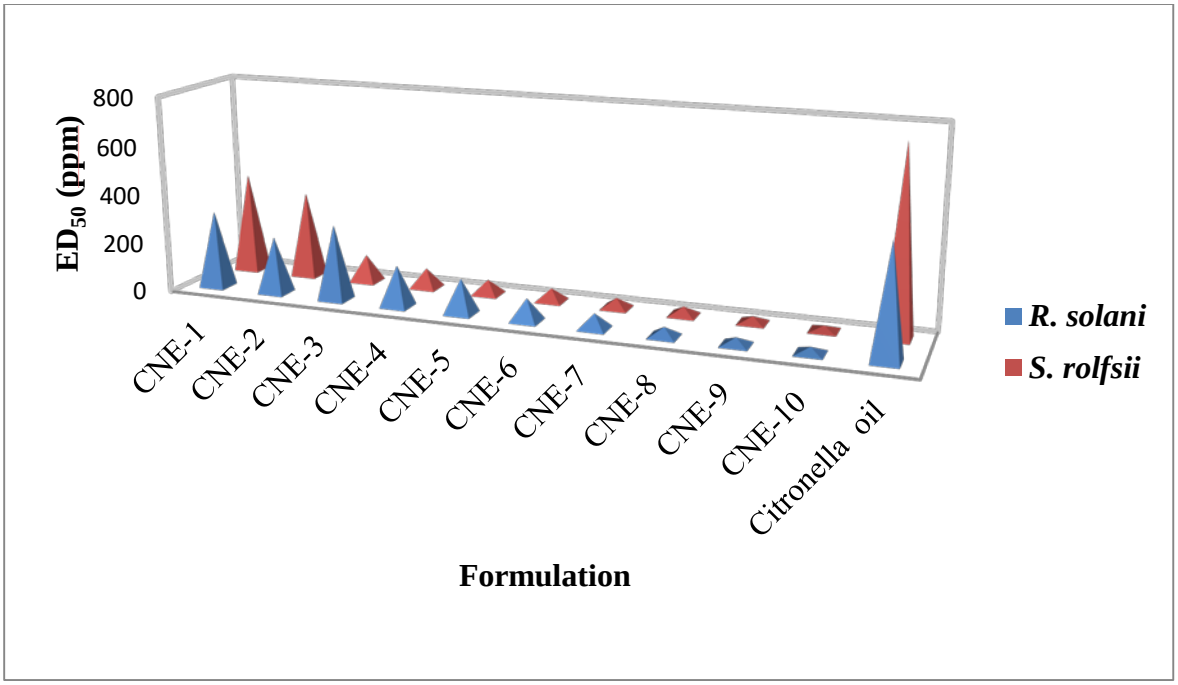


Figure-4.19 ED_{50} of citronella nanoemulsions

4.9 Larvicidal activity of neem oil nanoemulsion against *Spodoptera litura* (Fab)

4.9.1 Larval and pupal mortality

Bioactivity of neem nanoemulsions were studied against 3rd instar larvae of *S. litura* through leaf dip method at three different concentrations (1.0, 1.50 and 2.0%). Larvae were allowed to feed on treated leaf and were counted as dead when they were unable to move even after pressing with a brush. During observation, some of the larvae were found unable to molt and died during the molting process, while some were not able to grow. The mortality of larvae increased (0.0 to 80.0%, 0.0 to 60.0% and 13.33 to 80.0%) at 24 hrs, 48 hrs and 72 hrs, respectively. Maximum larval mortality (80%) occurred in NNE-7, NNE-8, NNE-9, and NNE-10 after 72 hrs followed by NNE-6 (66.67%) and NNE-5 (60.0%) whereas, remaining concentrations showed the mortality less than 50.0%.

During the study, pupal mortality was recorded by counting dead pupae which were darker in color and small in size. Pupal mortality ranged from 0.0 to 6.67% (Table 4.9). Maximum (6.67%) pupal mortality was observed at NNE-7, NNE-8, NNE-9 and NNE-10.

For larval weight reduction, larvae were weighted after 24 hrs, 48 hrs and 72 hrs. Effect of neem nanoemulsions on the weight reduction of *S. litura* at 24 hrs, 48

hrs and 72 hrs ranged from 49.67 to 88.0%, 50.33 to 91.0% and 52.33 to 90.33%, respectively (Table 4.9). NNE-10 showed the highest larval weight reduction (91.0%) at 2% concentration after 48 hrs, while minimum larval weight reduction was observed in NNE-1 (49.67%) at 1.00% concentration after 24 hrs (Table 4.9).

The adult emergence was found to be 86.67 and 0.0% in NNE-1 and NNE-10, respectively as against 86.67% in control. But only 1.33% abnormal malformed adult were noticed in NNE-7, NNE-8, NNE-9 and NNE-10. In NNE-1 at1.0 and 1.5% concentrations, 86.67% normal adult emergence occurred with 13.33% IGR activity. While using NNE-10, 100% IGR activity was observed. It was found that abnormal adults were formed only at a higher concentration of citronella oil (>3.00%) in nanoemulsion.

Table 4.9 Effect of NNEs on mortality and weight reduction of 3rd instar larvae of *S. litura*

Formulations	Conc (%)	Larval mortality (%)			Larval weight reduction (%)			Pupal mortality (%)	Adult emergence (%)	Malformation (%)	IGRA. (%)
		24hrs	48hrs	72hrs	24hrs	48hrs	72hrs				
NNE-1	1.00	0.00^{f} (0.00^{f})	0.00^{b} (0.00^{b})	13.33^{hi} (17.71^{gh})	49.67^{s} (44.81^{q})	50.33^{t} (45.19^{u})	52.33^{o} (46.34^{s})	0.00 (0.00)	86.67^{ab} (72.29^{abc})	0.00^{b} (0.00^{b})	13.33
	1.50	0.00^{f} (0.00^{f})	0.00^{b} (0.00^{b})	13.33^{hi} (17.71^{gh})	53.00^{rs} (46.72^{pq})	53.00^{t} (46.72^{tu})	54.00^{o} (47.29^{s})	0.00 (0.00)	86.67^{ab} (72.29^{abc})	0.00^{b} (0.00^{b})	13.33
	2.00	0.00^{f} (0.00^{f})	6.67^{ab} (8.86^{ab})	20.00G^{hi} (21.93^{fg})	55.00^{qr} (47.87^{pq})	55.67st (48.25^{stu})	56.00^{no} (48.45^{rs})	0.00 (0.00)	80.00^{abc} (68.07^{abcd})	0.00^{b} (0.00^{b})	20.00
NNE-2	1.00	0.00^{f} (0.00^{f})	6.67^{ab} (8.86^{ab})	20.00G^{hi} (21.93^{fg})	58.00^{pq} (49.61^{op})	59.00^{rs} (50.19^{rst})	59.33^{n} (50.38^{qr})	0.00 (0.00)	80.00^{abc} (68.07^{abcd})	0.00^{b} (0.00^{b})	20.00
	1.50	0.00^{f} (0.00^{f})	6.67^{ab} (8.86^{ab})	26.67F^{gh} (30.79^{efg})	61.00^{op} (51.36^{no})	62.00^{qr} (51.95^{qrs})	63.67^{m} (52.93^{pq})	0.00 (0.00)	73.33^{abcd} (59.21^{abcdef})	0.00^{b} (0.00^{b})	26.67
	2.00	0.00^{f} (0.00^{f})	13.33^{ab} (17.71^{a})	26.67f^{gh} (30.79^{efg})	61.67^{nop} (51.75^{mno})	62.00^{qr} (51.95^{qrs})	64.33^{lm} (53.34^{p})	0.00 (0.00)	73.33^{abcd} (59.21^{abcdef})	0.00^{b} (0.00^{b})	26.67
NNE-3	1.00	0.00^{F} (0.00^{f})	13.33^{ab} (17.71^{a})	33.33^{efgh} (35.01^{defg})	62.67^{mnop} (52.34^{mno})	63.33^{pqr} (52.73^{pqr})	64.67^{lm} (53.53^{p})	0.00 (0.00)	66.67^{bcde} (54.99^{bcdefg})	0.00^{b} (0.00^{b})	33.33
	1.50	0.00^{f} (0.00^{f})	13.33^{ab} (17.71^{a})	26.67^{fgh} (30.79^{efg})	64.67^{lmno} (53.53^{lmn})	64.67^{pq} (53.53^{opqr})	65.00^{lm} (53.73^{op})	0.00 (0.00)	73.33^{abcd} (63.85^{abcde})	0.00^{b} (0.00^{b})	26.67
	2.00	0.00^{f} (0.00^{f})	13.33^{ab} (17.71^{ab})	40.00^{defg} (39.23^{cdef})	66.00^{klmn} (54.33^{klmn})	66.67^{opq} (54.74^{nopq})	66.00^{klm} (54.33^{op})	0.00 (0.00)	60.00^{cde} ^{f}(50.77^{cdefg})	0.00^{b} (0.00^{b})	40.00
NNE-4	1.00	0.00^{f} (0.00^{f})	13.33^{ab} (17.71^{ab})	40.00^{defg} (38.86^{cdef})	66.67^{jklm} (54.74^{jklm})	67.33^{opq} (55.14^{mnopq})	67.67^{kl} (55.35^{nop})	0.00 (0.00)	60.00^{cdef} ($^{}$51.14^{cdefg})	0.00^{b} (0.00^{b})	40.00
	1.50	6.67^{f} (8.86^{ef})	20.00^{ab} (21.93^{ab})	46.67^{cdef} (43.08^{bcde})	68.00^{jkl} (55.56^{jkl})	68.67^{nop} (55.96^{lmnop})	69.00^{jk} (56.17^{mno})	0.00 (0.00)	53.33^{defg} (46.92^{defg})	0.00^{b} (0.00^{b})	46.67
	2.00	6.67^{f} (8.86^{ef})	20.00^{ab} (21.93^{ab})	46.67^{cdef} (43.08^{bcde})	70.67^{ijk} (57.21^{ijk})	70.67^{mno} (57.21^{klmno})	71.67^{ij} (57.84^{lmn})	0.00 (0.00)	40.00^{fg} (39.23^{fg})	0.00^{b} (0.00^{b})	60.00

NNE-5	1.00	6.67^{f} (8.86^{ef})	26.67^{ab} (26.15^{ab})	53.33^{bcde} (46.92^{bcde})	71.00^{ij} (57.43^{ij})	72.00^{lmno} (58.06^{klmn})	73.00^{hi} (58.70^{klm})	0.00 (0.00)	46.67^{efg} (43.08^{efg})	0.00^{b} (0.00^{b})	53.33
	1.50	13.33^{ef} (17.71^{def})	26.67^{ab} (26.15^{ab})	53.33^{bcde} (46.92^{bcde})	73.00^{hi} (58.70^{hi})	73.00^{klmn} (58.70^{jklm})	73.67^{ghi} (59.15^{jkl})	0.00 (0.00)	40.00^{fg} (39.23^{fg})	0.00^{b} (0.00^{b})	60.00
	2.00	13.33^{ef} (17.71^{def})	26.67^{ab} (26.15^{ab})	60.00^{abcd} (50.77^{abcde})	73.33^{hi} (58.92^{hi})	74.00^{jklmn} (59.35^{ijkl})	74.33^{ghi} (59.58^{jkl})	0.00 (0.00)	40.00^{fg} (39.23^{fg})	0.00^{b} (0.00^{b})	60.00
NNE-6	1.00	13.33^{ef} (17.71^{def})	26.67^{ab} (26.15^{ab})	60.00^{abcd} (51.14a^{bcde})	75.00^{ghi} (60.00^{ghi})	75.67^{ijklm} (60.45^{hijk})	76.67^{fgh} (61.$^{12ijk)}$)	0.00 (0.00)	40.00^{fg} (38.86^{fg})	0.00^{b} (0.00^{b})	60.00
	1.50	13.33^{ef} (17.71^{def})	26.67^{ab} (26.15^{ab})	66.67^{abc} (54.99^{abcd})	75.33^{ghi} (60.22^{ghi})	76.33^{hijkl} (60.89^{ghijk})	77.33^{fg} (61.57^{ij})	0.00 (0.00)	33.33^{g} (35.01^{g})	0.00^{b} (0.00^{b})	66.67
	2.00	13.33^{ef} (17.71^{def})	26.67^{ab} (26.15^{ab})	66.67^{abc} 54.99^{abcd})	77.00^{fgh} (61.35^{fgh})	78.00^{ghijk} (62.03^{fghij})	80.00^{ef} (63.44^{hi})	0.00 (0.00)	33.33^{g} (35.01^{g})	0.00^{b} (0.00^{b})	66.67
NNE-7	1.00	20.00^{def} (26.57^{cdef})	26.67^{ab} (26.15^{ab})	73.33^{ab} (59.21^{abc})	78.33^{efg} (62.27^{fg})	79.00^{ghij} (62.73^{fghi})	81.33^{de} (64.41^{gh})	6.67 (8.86)	2.00^{h} (6.56^{h})	0.00^{b} (0.00^{b})	98.00
	1.50	20.00^{def} (26.57^{cdef})	26.67^{ab} (26.15^{ab})	73.33^{ab} (59.21^{abc})	78.67^{efg} (62.50^{efg})	80.00^{fghi} (63.45^{efgh})	81.67^{de} (64.66^{fgh})	6.67 (8.86)	1.33^{h} (5.42^{h})	6.67^{ab} (8.86^{ab})	98.67
	2.00	20.00^{def} (26.57^{cdef})	26.67^{ab} (26.15^{ab})	73.33^{ab} (59.21^{abc})	80.33^{def} (63.68^{def})	81.00^{efghi} (64.16^{efgh})	82.00^{de} (64.90^{fgh})	6.67 (8.86)	6.67^{h} (8.86^{h})	1.33^{b} (5.42^{b})	93.33
NNE-8	1.00	20.00^{def} (26.57^{cdef})	33.33^{ab} (30.00^{ab})	80.00^{a} (63.43^{ab})	80.67^{cdef} (63.92^{def})	81.67^{defgh} (64.65^{defg})	82.67^{de} (65.40$^{fgh)}$	6.67 (8.86)	0.00^{h} (0.00^{h})	1.33^{b} (5.42^{b})	100.00
	1.50	33.33^{cde} (35.01^{bcde})	40.00^{ab} (34.22^{ab})	80.00^{a} (68.07^{a})	82.67^{bcde} (65.43^{cde})	82.67^{cdefg} (65.43^{cdef})	84.00^{cd} (66.43^{efg})	6.67 (8.86)	0.00^{h} (0.00^{h})	1.33^{b} (3.85^{b})	100.00
	2.00	40.00^{cd} (39.23^{abcd})	33.33^{ab} (30.00^{ab})	80.00^{a} (63.43^{ab})	82.67^{bcde} (65.41^{cde})	85.00^{bcdef} (67.22^{bcde})	85.00^{bcd} (67.22^{def})	6.67 (8.86)	0.00^{h} (0.00^{h})	13.33^{a} (17.71^{a})	100.00
NNE-9	1.00	46.67^{c} (43.08^{abcd})	33.33^{ab} (30.00^{ab})	80.00^{a} (63.43^{ab})	84.00^{abcd} (66.43^{bcd})	86.33^{abcde} (68.32^{bcd})	86.67^{abc} (68.60^{cde})	6.67 (8.86)	0.00^{h} (0.00^{h})	13.33^{a} (17.71^{a})	100.00
	1.50	46.67^{c} (43.08^{abcd})	40.00^{ab} (33.85^{ab})	80.00^{a} (68.07^{a})	85.00^{abcd} (67.22^{abc})	86.33^{abcde} (68.32^{bcd})	87.33^{abc} (69.15^{bcd})	6.67 (8.86)	0.00^{h} (0.00^{h})	1.33^{b} (3.85^{b})	100.00
	2.00	53.33^{bc} (46.92^{abc})	46.67^{ab} (38.07^{ab})	80.00^{a} (63.43^{ab})	85.33^{abc} (67.48^{abc})	87.33^{abc} (69.19^{abc})	87.33^{abc} (69.40^{abcd})	6.67 (8.86)	0.00^{h} (0.00^{h})	1.33^{b} (5.42^{b})	100.00

NNE-10	1.00	73.33^{ab} (59.21^{ab})	53.33^{ab} (42.29^{ab})	80.00^{a} (63.43^{ab})	86.00^{ab} (68.04^{abc})	87.00^{abcd} (68.93^{abc})	88.33^{ab} (70.03^{abc})	6.67 (8.86)	0.00^{h} (0.00^{h})	1.33^{b} (5.42^{b})	100.00
	1.50	80.00^{a} (63.43^{a})	53.33^{ab} (42.29^{ab})	80.00^{a} (63.43^{ab})	87.00^{ab} (68.87^{ab})	89.33^{ab} (70.94^{ab})	89.67^{a} (71.25^{ab})	6.67 (8.86)	0.00^{h} (0.00^{h})	1.33^{b} (5.42^{b})	100.00
	2.00	80.00^{a} (63.43^{a})	60.00^{a} (51.14^{a})	80.00^{a} (63.43^{ab})	88.00^{a} (69.73^{a})	91.00^{a} (72.56^{a})	90.33^{a} (71.89^{a})	6.67 (8.86)	0.00^{h} (0.00^{h})	1.33^{b} (5.42^{b})	100.00
NE	1.00	0.00^{f} (0.00^{f})	0.00^{b} (0.00^{b})	0.00^{i} (0.00^{h})	-	-	-	0.00 (0.00)	86.67^{ab} (76.92^{ab})	0.00^{b} (0.00^{b})	13.33
	1.50	0.00^{f} (0.00^{f})	0.00^{b} (0.00^{b})	0.00^{i} (0.00^{h})	-	-	-	0.00 (0.00)	93.33^{a} (81.14^{a})	0.00^{b} (0.00^{b})	6.67
	2.00	0.00^{f} (0.00^{f})	0.00^{b} (0.00^{b})	0.00^{i} (0.00^{h})	-	-	-	0.00 (0.00)	93.33^{a} (81.14^{a})	0.00^{b} (0.00^{b})	6.67
p-value		<.0001 (<.0001)	<.0001 (<.0001)	<.0001 (<.0001)	<.0001 (<.0001)	<.0001 (<.0001)	<.0001 (<.0001)	0.8191 (0.8191)	<.0001 (<.0001)	0.0007 (0.0025)	
CV (%)		35.28 39.06)	68.39 (58.17)	22.54 (21.90)	1.77 (1.43)	2.00 (1.74)	2.28 (2.01)	269.87 (269.87)	28.04 (29.53)	271.01 (208.77)	
SE(d)		5.412 (5.942	12.861 (10.452)	9.220 (7.773)	1.050 (0.689)	1.204 (0.849)	1.385 (0.987)	5.342 (7.095)	9.367 (8.742)	2.950 (4.364)	
LSD at 1%		24.392 (26.78)	57.962 (47.105)	24.479 (20.637)	4.7082 (3.0915)	5.4026 (3.8072)	3.6884 (2.628)	NS (NS)	24.868 (23.209)	7.8327 (11.586)	

* Values given are the mean of three replicates in % and arc sine transformed values in parenthesis followed by same letter (s) are not significantly different.

4.10 Larvicidal activity of Citronella oil nanoemulsions against *Spodoptera litura* (Fab)

Larval and pupal mortality

Bioactivity of citronella nanoemulsions were also studied against 3rd instar larvae of *S. litura* through leaf dip method at three different concentrations (1.0, 1.5 and 2.0%). The mortality of larvae increased (0.0 to 66.67%, 0.0 to 66.67% and 6.67 to 73.33%) at 24 hrs, 48 hrs and 72 hrs, respectively. Maximum (73.33%) larval mortality occurred in CNE-8, CNE-9, and CNE-10 after 72 hrs followed by CNE-7, CNE-6 (60.0%) and CNE-5 (53.33%) whereas; remaining concentrations showed the mortality less than 50.0%.

Pupal mortality also ranged from 0.0 to 6.67% (Table 4.10). Maximum pupal mortality (6.67%) was observed in CNE-8, CNE-9 and CNE-10.

The mean per cent larval weight reduction at 24 hrs, 48 hrs and 72 hrs ranged from 49.33 to 87.67%, 49.67 to 88.33% and 50.0 to 97.67%, respectively (Table 15). CNE-10 showed the highest larval weight reduction (97.67%) at 2% concentration after 72 hrs, while minimum larval weight reduction was observed in CNE-1 (49.33%) at 1.00% concentration after 24 hrs (Table 4.10).

The adult emergence was found to be between 93.33 and 13.33% in CNE-1 and CNE-10, respectively while 86.67% in control. But only 6.67% abnormal malformed adult was noticed in CNE-8, CNE-9 and CNE-10. In CNE-1 at 1.0 and 1.5% concentrations, 93.33% normal adult emergence occurred with 6.67% IGR activity. While using NNE-10, 86.67% IGR activity was observed. It was found that abnormal adults were formed only at higher concentration of neem oil (> 4.00%) in citronella nanoemulsion.

Table 4.10 Effect of Citronella nanoemulsions (CNEs) on mortality and weight reduction of 3rd instar larvae of *S. litura*

Formulations	Conc. (%)	larval mortality (%)			Larval Weight reduction (%)			Percent pupal mortality at 7 DAT	Normal adult (%)	Malformation (%)	IGRA. (%)
		24hrs	48hrs	72hrs	24hrs	48hrs	72hrs				
CNE-1	1.00	0.00^{e} (0.00^{e})	0.00^{g} (0.00^{e})	6.67^{ef} (8.86^{cd})	49.33^{s} (44.62^{q})	49.67^{q} (44 .81^{p})	50.00^{q} (45.00^{r})	0.00 (0.00)	93.33^{a} (81.14^{a})	0.00 (0.00)	6.67
	1.50	0.00e (0.00e)	0.00g (0.00e)	6.67ef (8.86cd)	51.67rs (45.96q)	52.00q (46.15p)	53.33pq (46.91qr)	0.00 (0.00)	93.33a (81.14a)	0.00 (0.00)	6.67
	2.00	0.00^{e} (0.00^{e})	6.67^{fg} (8.86^{de})	13.33^{def} (17 .71^{bcd})	54.67^{qrs} (47.68^{pq})	55.00^{pq} (47 .87^{op})	56.00^{opq} (48.45^{pqr})	0.00 (0.00)	86.67^{ab} (72.29^{ab})	0.00 (0.00)	13.33
CNE-2	1.00	0.00^{e} (0.00^{e})	13.33^{efg} (17.71^{cd})	13.33^{def} (17 .71^{bcd})	57.00^{pqr} (49.04^{opq})	60.67^{op} (51 .16no)	61.33^{nop} (51.55^{opqr})	0.00 (0.00)	86.67^{ab} (72.29^{ab})	0.00 (0.00)	13.33
	1.50	0.00^{e} (0.00^{e})	13.33 (17.71^{cd})	20.00^{cdef} (26 .57^{abcd})	60.67^{opq} (51.16^{nop})	62.00^{no} (5 1.95^{mno})	63.33^{mno} (52.7 4^{nopqr})	0.00 (0.00)	80.00^{abc} (63.43^{ab})	0.00 (0.00)	20.00
	2.00	0.00^{e} (0.00^{e})	13.33^{efg} (17.71^{cd})	26.67^{bcdef} (3 0.79^{abcd})	62.33^{nop} (52.15^{mnop})	64.00^{no} (53 .13^{lmn})	65.00^{lmn} (53.73 ^{mnopq})	0.00 (0.00)	73.33^{abcd} (59.21^{ab})	0.00 (0.00)	26.67
CNE-3	1.00	0.00^{e} (0.00^{e})	13.33^{efg} (17.71^{cd})	33.33^{abcdef} (35.01^{abcd})	62.67^{nop} (52.34^{lmno})	64.67^{mno} (5 3.53^{klmn})	65.67^{lmn} (54.13 ^{mnopq})	0.00 (0.00)	66.67^{abcd} (54.99^{abc})	0.00 (0.00)	33.33
	1.50	0.00^{e} (0.00^{e})	13.33^{efg} (17.71^{cd})	40.00^{abcdef} (39.23^{abcd})	64.00^{mnop} (53.13^{klmno})	66.00^{lmno} (5 4.33^{klmn})	68.33^{klmn} (55.7 6^{lmnop})	0.00 (0.00)	60.00^{abcd} (50.77^{abc})	0.00 (0.00)	40.00
	2.00	0.00^{e} (0.00^{e})	13.33^{efg} (17.71^{cd})	40.00^{abcdef} (39.23^{abcd})	65.33^{mno} (53.93^{klmn})	67.33^{klmn} (5 5.14^{jklmn})	70.00^{jklm} (56.7 9^{klmno})	0.00 (0.00)	60.00^{abcd} (50.77^{abc})	0.00 (0.00)	40.00
CNE-4	1.00	0.00^{e} (0.00^{e})	20.00^{defg} (26.57^{bcd})	40.00^{abcdef} (39.23^{abcd})	66.67^{lmno} (54.74^{jklmn})	68.00^{jklmn} (55.55^{jklm})	71.00^{ijklm} (57.4 2^{klmno})	0.00 (0.00)	60.00^{abcd} (50.77^{abc})	0.00 (0.00)	40.00
	1.50	0.00^{e} (0.00^{e})	20.00^{defg} (26.57^{bcd})	40.00^{abcdef} (39.23^{abcd})	69.00^{klmn} (5 6.17^{ijklm})	70.33^{ijklm} (5 7.01^{ijkl})	72.33^{ijkl} (58.27^{j} ^{klmno})	0.00 (0.00)	60.00^{abcd} (50.77^{abc})	0.00 (0.00)	40.00
	2.00	0.00^{e} (0.00^{e})	20.00^{defg} (26.57^{bcd})	46.67^{abcde} (4 3.08^{abcd})	70.00^{jklm} (5 6.79^{hijkl})	70.67^{hijklm} (57.21^{ijkl})	73.33^{hijkl} (58.9 2^{ijklmno})	0.00 (0.00)	53.33^{bcd} (46.92^{abc})	0.00 (0.00)	46.67

CNE-5	1.00	0.00^{e} (0.00^{e})	20.00^{defg} (26.57^{bcd})	46.67^{abcde} (43.08^{abcd})	70.67^{ijklm} (57.21^{ghijk})	71.00^{hijkl} (57.42^{hijk})	74.67^{ghijk} (59.79^{ijklmn})	0.00 (0.00)	53.33^{bcd} (46.92^{abc})	0.00 (0.00)	46.67
	1.50	0.00^{e} (0.00^{e})	20.00^{defg} (26.57^{bcd})	53.33^{abcd} (46.92^{abc})	72.67^{hijkl} (58.48^{fghij})	73.00^{ghijk} (58.70^{ghij})	76.33^{fghijk} (60.94^{ijklm})	0.00 (0.00)	46.67^{cde} (43.08^{abc})	0.00 (0.00)	53.33
	2.00	6.67^{de} (8.86^{de})	20.00^{defg} (26.57^{bcd})	60.00^{abc} (50.77^{abc})	73.00^{hijkl} (58.70^{fghij})	74.00^{ghij} (59.35^{ghij})	77.00^{fghij} (61.37^{hijklm})	0.00 (0.00)	40.00^{de} (39.23^{bc})	0.00 (0.00)	60.00
CNE-6	1.00	6.67^{de} (8.86^{de})	20.00^{defg} (26.57^{bcd})	60.00^{abc} (50.77^{abc})	74.67^{ghijk} (59.78^{efghi})	75.67^{fghi} (60.46^{fghi})	78.67^{fghi} (62.50^{ghijkl})	0.00 (0.00)	40.00^{de} (39.23^{bc})	0.00 (0.00)	60.00
	1.50	13.33^{cde} (17.71^{cde})	26.67^{def} (30.79^{abcd})	60.00^{abc} (50.77^{abc})	75.33^{fghijk} (60.22^{efghi})	76.67^{efgh} (61.12^{efghi})	81.67^{efgh} (64.69^{fghijk})	0.00 (0.00)	40.00^{de} (39.23^{bc})	0.00 (0.00)	60.00
	2.00	13.33^{cde} (17.71^{cde})	33.33^{cde} (35.01^{abc})	60.00^{abc} (50.77^{abc})	76.67^{efghij} (61.12^{defgh})	77.33^{efg} (61.58^{efgh})	82.67^{defg} (65.42^{efghij})	0.00 (0.00)	40.00^{de} (39.23^{bc})	0.00 (0.00)	60.00
CNE-7	1.00	13.33^{cde} (17.71^{cde})	33.33^{cde} (35.01^{abc})	60.00^{abc} (50.77^{abc})	77.00^{efghij} (61.35^{defg})	78.67^{defg} (62.50^{efg})	84.33^{def} (66.73^{efghi})	0.00 (0.00)	40.00^{de} (39.23^{bc})	0.00 (0.00)	60.00
	1.50	13.33^{cde} (17.71^{cde})	33.33^{cde} (35.01^{abc})	60.00^{abc} (50.77^{abc})	77.67^{defghi} (61.81^{cdef})	80.33^{cdef} (63.69^{def})	87.33^{cde} (69.19^{defgh})	0.00 (0.00)	40.00^{de} (39.23^{bc})	0.00 (0.00)	60.00
	2.00	20.00^{cde} (26.57^{bcd})	33.33^{cde} (35.01^{abc})	60.00^{abc} (50.77^{abc})	78.33^{cdefgh} (62.27^{cdef})	80.67^{bcdef} (63.96^{cdef})	88.33^{bcde} (70.04^{defg})	0.00 (0.00)	40.00^{de} (39.23^{bc})	0.00 (0.00)	60.00
CNE-8	1.00	20.00^{cde} (26.57^{bcd})	40.00^{bcd} (39.23^{abc})	66.67^{ab} (54.99^{ab})	80.33^{bcdefg} (63.69^{bcde})	81.00^{bcdef} (64.22^{cdef})	88.00^{bcde} (69.74^{defg})	6.67 (8.86)	13.33^{e} (17.71^{c})	6.67 (8.86)	86.67
	1.50	20.00^{cde} (26.57^{bcd})	40.00^{bcd} (39.23^{abc})	73.33^{a} (63.85^{a})	82.33^{abcdef} (65.15^{abcd})	82.33^{abcde} (65.17^{bcde})	89.67^{abcde} (71.28^{cdef})	6.67 (8.86)	13.33^{e} (17.71^{c})	6.67 (8.86)	86.67
	2.00	20.00^{cde} (26.57^{bcd})	40.00^{bcd} (39.23^{abc})	73.33^{a} (63.85^{a})	83.33^{abcde} (65.91^{abc})	84.67^{abcd} (66.96^{abcd})	90.67^{abcd} (72.85^{bcde})	6.67 (8.86)	13.33^{e} (17.71^{c})	6.67 (8.86)	86.67
CNE-9	1.00	20.00^{cde} (26.57^{bcd})	53.33^{abc} (46.92^{ab})	73.33^{a} (63.85^{a})	83.67^{abcde} (66.16^{abc})	85.33^{abc} (67.50^{abcd})	93.33^{abc} (75.24^{abcd})	6.67 (8.86)	13.33^{e} (17.71^{c})	6.67 (8.86)	86.67
	1.50	26.67^{bcd} (30.79^{abcd})	60.00^{ab} (50.77^{ab})	73.33^{a} (63.85^{a})	84.67^{abcd} (66.96^{ab})	85.67^{abc} (67.76^{abcd})	93.67^{abc} (75.57^{abcd})	6.67 (8.86)	13.33^{e} (17.71^{c})	6.67 (8.86)	86.67
	2.00	33.33^{bc} (35.01^{abc})	60.00^{ab} (50.77^{ab})	73.33^{a} (63.85^{a})	85.00^{abc} (67.22^{ab})	86.00^{abc} (68.04^{abc})	96.00^{ab} (78.52^{abc})	6.67 (8.86)	13.33^{e} (17.71^{c})	6.67 (8.86)	86.67

CNE-10	1.00	46.67^{ab} (43.08^{abc})	60.00^{ab} (50.77^{ab})	73.33^{a} (63.85^{a})	85.33^{abc} (67.51^{ab})	86.67^{ab} (68.60^{ab})	96.00^{ab} (78.72^{abc})	6.67 (8.86)	13.33^{e} (17.71^{c})	6.67 (8.86)	86.67
	1.50	60.00^{a} (50.77^{ab})	60.00^{ab} (50.77^{ab})	73.33^{a} (63.85^{a})	86.33^{ab} (68.31^{a})	87.67^{a} (69.44^{a})	97.00^{a} (80.65^{ab})	6.67 (8.86)	13.33^{e} (17.71^{c})	6.67 (8.86)	86.67
	2.00	66.67^{a} (54.99^{a})	66.67^{a} (54.99^{a})	73.33^{a} (63.85^{a})	87.67^{a} (69.46^{a})	88.33^{a} (70.05^{a})	97.67^{a} (81.53^{a})	6.67 (8.86)	13.33^{e} (17.71^{c})	6.67 (8.86)	86.67
CE	1.00	0.00^{e} (0.00^{e})	0.00^{g} (0.00^{e})	0.00^{f} (0.00^{d})	-	-	-	6.67 (8.86)	86.67^{ab} (76.92^{ab})	6.67 (8.86)	13.33
	1.50	0.00^{e} (0.00^{e})	0.00^{g} (0.00^{e})	0.00^{f} (0.00^{d})	-	-	-	6.67 (2.71)	86.67^{ab} (72.29^{ab})	6.67 (8.86)	13.33
	2.00	0.00^{e} (0.00^{e})	0.00^{g} (0.00^{e})	0.00^{f} (0.00^{d})	-	-	-	6.67 (8.86)	86.67^{ab} (72.29^{ab})	6.67 (8.86)	13.33
p-value		<.0001 (<.0001)	<.0001 (<.0001)	<.0001 (<.0001)	<.0001 (<.0001)	<.0001 (<.0001)	<.0001 (<.0001)	0.74 (0.76)	<.0001 (<.0001)	0.7424 (0.7424)	
CV (%)		52.28 (52.99)	24.87 (24.95)	27.11 (29.31)	2.77 (2.12)	2.26 (1.94)	2.97 (3.42)	273.00 (269.70)	19.17 (23.91)	258.90 (258.90)	
SE(d)		5.174 (5.717)	5.334 (5.523)	10.063 (9.838)	1.633 (1.013)	1.356 (0.942)	50.00^{q} (45.00^{r})	4.998 (6.681)	7.747 (8.757)	5.125 (6.807)	
LSD at 1%		23.317 (25.765)	24.039 (24.89)	45.352 (44.338)	7.323 (4.545)	6.0828 (4.2267)	53.33^{pq} (46.91^{qr})	Ns (ns)	34.914 (39.465)	ns (ns)	

* Values given are the mean of three replicates in % and arc sine transformed values in parenthesis followed by same letter (s) are not significantly different

4. DISCUSSONS

The primary oil-in-water (O/W) emulsion of neem oil was prepared using Tween-20, neem oil and distilled water as ingredients while citronella oil, Triton-X 100 and distilled water were used for the preparation of (O/W) primary emulsion of citronella oil. The best ratio of oil, surfactant and distilled water for making primary emulsions of both the oils was 0.5:1.0:8.5. It was found that the increase in surfactant to neem oil or citronella oil ratio caused a decrease in droplet size (Tables 4.1, 4.2 and Figure-4.1). Nanoemulsions are known as isotropic, thermodynamically stable, transparent system of oil; water and surfactant with a droplet size usually in the range of 10-100 nm (Shafiq *et al*., 2007; Mei *et al*., 2003). The micelle size of the nanoemulsions was determined using DLS. In DLS analysis, the average micelle size of neem nanoemulsions ranged from 11.23±3.86 to 17.80±4.52 nm and that of citronella nanoemulsions from 8.12±2.80 to 12.04±3.74 nm for citronella nanoemulsions (Figures-4.2, 4.3 and 4.4), below the reported size range of similar nanoemulsions (Sakulku *et al*., 2009; Jerobin *et al*., 2012; Agrawal *et al*., 2017). The very low size range of nanomicelle of the present nanoemulsion formulation may be attributed to the use of Tween-20 and Triton-X-100 as emulsifiers, where as in the previous studies use of Tween-80, SPAN-80, poly (ethylene glycols), and other additives like glycerol, sodium alginates and starch (Sakulku *et al*., 2009; Jerobin *et al*., 2012; Agrawal *et al*., 2017) were reported. Triton-X-100 is a non-ionic surfactant, where poly (ethylene glycols) is attached with p-(1, 1, 3, 3-tetramethylbutyl)-phenyl group through ether linkage. The presence of the strong hydrophobic moiety (p-(1, 1, 3, 3-tetramethylbutyl)-phenyl group) in PEG chain of Triton-X-100 may be attributed to the occurrence of very low size of developed nanomicelle. Previously, Triton-X-100 was used in the development of nanoemulsion of α-tocopherol, cetylpyridinium chloride (Hwang *et al*., 2013; Saxena *et al*., 2017). Tween-20 was used as surfactant, because non-ionic surfactants are known to be less affected by pH and ionic strength. Increase in surfactant concentration in the emulsion leads to reduced droplet size and emulsion turbidity as emulsion turbidity is a function of particle concentration and the size of particle (Reddy and Fogler, 1981).

The spherical form of developed nanoemuslion as revealed by Transmission Electron Microscope (TEM) (Figures-4.5 and 4.6) is similar to those obtained by Mao *et al*., (2009), who reported spherical morphology of β-carotene nanoemulsion

stabilised by Tween-20. Another study by Baboota *et al.*, (2007) showed that celecoxib nanoemulsion exhibited size range between 19 and 78 nm by TEM studies. Reduction in droplet size increases the surface area of the droplet. This results in increasing the rate of accumulation of a.i on fungi and larvae causing increased fungicidal and larvicidal efficacy of the nanoemulsion.

The FT-IR spectra of citronella oil, neem oil, Triton-X-100 and nanoemulsion are shown in Figure-4.7. Significant bands corresponding to important functional groups are listed in (Table 4.3). Citronella oil showed major bands at 3365, 2925, 1724 and 1669 cm^{-1} corresponding to OH stretching, methylene C-H asymmetric stretching, carbonyl stretching and olefinic unsaturation stretching, respectively (Figure-4.7 A). These bands were due to presence of citronellal, citronellol, geraniol as major constituents in citronella oil (Wany *et al.*, 2014). The characteristic bands of neem oil were observed at 2922, 2852, 1744, 1464 and 1160 cm^{-1} corresponding to OH stretching, aromatic/vinyl C-H stretching, aliphatic C-H stretching, carbonyl stretching and ether stretching, respectively (Jerobin *et al.*, 2012), (Figure-4.7, B). The developed nanoemulsions showed characteristics bands of both citronella oil and neem oil (Figures-4.7, D). The characteristic bands of Triton-X-100 were not clear in the FT-IR spectra of nanoemulsion due to similarity of functional groups with oils (Figure-4.7, C).

High degree of stability of neem nanoemulsions was observed according to CIPAC method. No foaming and separation of oily matter was seen in developed neem nanoemulsions even after 24 hrs (Table 4.4 and Figure-4.8). Also, citronella nanoemulsions formed stable, nearly transparent or translucent colloidal system, on dilution with standard hard water D (Table 4.5 and Figure-4.9). The emulsion stability on standing was checked and it was found that no free oil, frothing or any cream was formed. Again on re-emulsification, no abnormality and separation of cream, froth and oil was observed. The stabilization of nano droplet in the emulsions with 0.5.0: 1.0:8.5 ratio of oil and surfactant would be due to surfactant, which reduces interfacial free energy and provides mechanical barrier to coalescence (Reiss, 1975).

pH plays an important role in the stability of the emulsion system (Karthik *et al.*, 2017). The pH of neem and citronella oils nanoemulsions measured are given in (Table 4.6). The pH values of neem nanoemulsions ranged from 3.53-4.51 and that of

citronella nanoemulsions from 2.30 to 5.85. No relation was found for the pH change with increasing concentrations of plant oils in nanoemulsions.

The agricultural crop loss due to insect pests, diseases, nematodes and weeds has been recorded even up to 50% in spite of the application of modern crop protection measures (Devakumar *et al.,* 2007). Of these, a considerable annual crop loss is due to plant diseases caused by phytopathogenic fungi, estimated up to about 20% and poses continuous challenge to modern agriculture disease management system. These phytopathogenic fungi infect seeds, seedlings and mature plants in the field causing many diseases such as collar rot, wilt, damping off and dry root rot (Aggarwal *et al.,* 2009). Among these fungi, *Rhizoctonia solani* and *Sclerotium rolfsii* infect many field crops.

At present, quick and effective management of most of plant pathogen fungi is generally achieved by the use of synthetic fungicides. In recent years, a large number of synthetic fungicides are banned in western world because of their undesirable attributes such as acute toxicity, long degradation periods, accumulation in food chain and toxicity to non- targeted organisms (Gatto *et al.,* 2011). However, the research of a new control system by botanical extract and bioagent is becoming a new possibility. Development of safe, effective and environment friendly botanical formulations has been thrust area of research since centuries as alternative strategy of pest management.

In the current study, different concentrations (7.8, 15.625, 31.25, 62.5, 125 and 250 ppm of neem and citronella nanoemulsions were screened for their antifungal activity against *R. solani* and *S. rolfsii* by poisoned food technique (Sharma and Tripathi, 2008). The neem nanoemulsions (NNEs) showed excellent fungicidal activity against *R. solani* and *S. rolfsii* (Table 4.7 and Figures-4.10, 4.11, 4.12 and 4.13). ED_{50} values were found to vary from 13.69 to 109.71 mg L^{-1} for *R. solani* and 14.71 to 85.97 mg L^{-1} for *S. rolfsii* (Figure.4.14). Results revealed that among the screened neem nanoemulsions, NNE-10 was found to be most effective against both *R. solani* (ED_{50} 13.69 mg L^{-1}) and *S. rolfsii* (ED_{50} 14.71 mg L^{-1}), but it has statistically insignificant difference with NNE-9. While the least antifungal activity against both *R. solani* (ED_{50} 109.71 mg L^{-1}) and *S. rolfsii* (ED_{50} 85.97 mg L^{-}) was shown in NNE-1. The results also showed that ED_{50} of neem nanoemulsions (NNEs) against *R. solani* and *S. rolfsii* decrease with increase in the amount of citronella oil

from (0.50 to 5%). The data further revealed that neem nanoemulsions showed significant fungicidal activity against, *R. solani* and *S. rolfsii* compared to neem oil. Our results were in agreement with Shivpuri and Gupta, 2001; Sarpeleh *et al.*, 2009.

Several compounds have been reported from neem tree and among these, azadirachtin-A, nimbin and salanin were the major triterpenoids having several biological activities Biswas *et al.,* 2002; Subapriya and Nagini, 2005). Azadirachtin is widely used in agriculture to combat insects, nematodes, fungi and bacteria. Wang *et al.,* (2010) reported that neem extracts showed antifungal properties. Singh *et al.*, (2015) reported that neem has demonstrated antifungal activity against fungi that cause certain plant diseases like rots, smuts, wilts, mildews, diebacks, blights and other plant diseases. Its extract is one of the most important plant products which inhibit mycotoxin production. The main chemicals of neem oil with antifungal activities are a mixture of triterpenoids and tetranortriterpenoid compounds. Neem leaf constituents are known to potentially inhibit aflatoxin production in *Aspergillus parasiticus* without affecting fungal growth (Ghorbanian *et al.,* 2008). Study on the effect of neem extracts on growth, sporulation, morhology and OTA production by *Penicillium verrucosum* and *P. brevicompactum* were investigated. The results show that neem extracts have fungitoxic activity, even though their mode of action is still not fully understood (Mossini *et al.,* 2009). Nimbin also demonstrated antifungal activity by inhibiting the growth of *Tinea rubrum* (Biswas *et al.,* 2002). Ghotbi *et al.*, (2014) reported that azadirachtin possess antifungal properties. Ali *et al.* (2015) reported that neem extract at 1000 ppm concentration shown inhibition of *R. solani* (47.62%). The antifungal activity of neem has been attributed to volatile sulfides, and the limonoid, gedumin (Iyer, 1991). Sehajpal *et al*., (2009) reported that *A. indica* gave moderate effect against *R. solani*, and its fungitoxic activity may be due to the present of azadirchtin containing desactylmbin and azadiradione, a chemical constituent in *A. indicia* oil is known to possess antifungal activity (Sehajpal *et al*., (2009).

Citronella nanoemulsions were evaluated for their antifungal activity against *R. solani* and *S. rolfsii* by the poisoned food technique and the ED_{50} values of different nanoemulsions are given in (Table 4.8). The citronella nanoemulsions (CNEs) showed excellent fungicidal activity against *R. solani* and *S. rolfsii* (Figures-4.15, 4.16, 4.17 and 4.18). The results showed that the ED_{50} of citronella nanoemulsions (CNEs)

against *R. solani* and *S. rolfsii* decreased with increasing amounts of neem oil (0.50 to 5%). ED_{50} values were statistically significant and varied from 25.64 to 311.04 mg L^{-1} for *R. solani* and 20.88 to 405.75 mg L^{-1} for *S. rolfsii* (Table 4.8). Table 4.19) shows that citronella nanoemulsion (CNE-10) was most active against both *R. solani* (ED_{50} 25.64 mg L^{-1}) and *S. rolfsii* (ED_{50} 20.88 mg L^{-1}), but statistically insignificant with CNE-9, while CNE-1was least active against both *R. solani* (ED_{50} 311.04 mg L^{-1}) and *S. rolfsii* (ED_{50} 405.75 mg L^{-1}). The data revealed that citronella nanoemulsion showed significant fungicidal activity against, *R. solani* and *S. rolfsii* compared to citronella oil. Results of current study were in agreement with many researchers. Li *et al.,* (2013) found that different citronella oil inhibited the growth of *Aspergillus niger*. Nogueira *et al.,* (2010) reported that the antifungal activity of essential oil might be related to their lipophilic characters which can penetrate the plasma membrane. Previous studies using the disc diffusion method had revealed that citronella oil exhibited antibacterial and fungicidal activity Nakahara *et al.,* (2003) reported that the antifungal assay using the vapor-agar contact method showed crude essential oil from *Cymbopogon nardus* suppressed the growth of several species of *Aspergillus*, *Penicillum* and *Eurotium* species at dose of 250 mg L^{-1} in air; the most active compounds were citronellal and linalool. It has also been reported that nanoemulsions hold promise as an antimicrobial agent (Lawrence and Rees, 2000).

According to the report of FAO, US $120 billion losses worldwide were caused by 20-40% decrease in crop yield, due to the attack from pathogenic organisms and insect pests (Suresh *et al.*, 2017). Among the different insect pests, *Spodoptera litura* (Noctuidae: Lepidoptera), is an economically important polyphagous insect in many agricultural and horticultural crops in many countries including India, Japan, China and Southeast Asia (Sahayaij *et al*., 2007). It also has a wide range of host, which belongs to about 40 plant families. It also causes loss of many economically important cultivated crops even up to 100% in field (Abbas *et al.,* 2014). It is reported to attack more than 200 different species of plants, of which 40 species are grown in India mainly, crucifers, cucurbits, groundnut, maize, castor, tea, tobacco, cotton, jute, lucerne, rice, soybean, cabbage, capsicum and potato including some ornamental plants (Abdul Razak *et al.,* 2014). Various insecticides have been used for management of *S. litura*. However, due to their indiscriminate use, it has developed multiple types of resistance, causing difficulties in controlling it in the field

(Abbas *et al.,* 2012). It has shown resistance against almost all the insecticide groups, such as, organochlorines, carbamates, organophosphates (OPs) and pyrethroids (Ahmad *et al.,* 2007) including new insecticides like lufenuron, fipronil, avermectins, indoxacarb, spinosad and insect growth regulators (Ahmad *et al.,* 2008). Research Worldwide since last few years is focused to overcome resistance problems using target-based biopesticides such as compounds based on bacteria, fungi, insect growth regulators and botanical pesticides. In the current study, an alternative solution for the management of *S. litura* has been envisaged wherein innovative botanical formulations, viz: nanoemulsions of botanical oils mixture containinig different ratio of neem and citronella oils were developed and evaluated against 3rd instar larvae of *S. litura* (Fab). The present investigation was carried out to ascertain the biological activity of neem and citronella oils nanoemulsions.

Bioactivity of neem nanoemulsions were studied against 3rd instar larvae of *S. litura* through leaf dip method at three different concentrations (1.0, 1.5 and 2.0%). The mortality of larvae was found to increase from 0.0 to 80.0%, 0.0 to 60.0% and 13.33 to 80.0% at 24 hrs, 48 hrs and 72 hrs, respectively (Table 4.9). Maximum (80.0%) larval mortality occurred at NNE-7, NNE-8, NNE-9 and NNE-10 after 72 hrs followed by NNE-6 (66.67%) and NNE-5 (60.0%). The remaining nanoemulsions showed less than 50.0% mortality (Table 4.9). These results indicated that when citronella oil was added to the neem oil nanoemulsion, the insecticidal activity of the formulation was enhanced which showed that the chemical compounds present in citronella oil may be responsible for the larvicidal activity. Pinheiro *et al.,* (2013) reported that essential oil of citronellal grass at 1.0% (w/v) caused significant mortality of *Myzus persicae* (96.9±1.57%). Citronellal, which is the major constituent of citronella oil, was reported to act synergistically with other natural complements, trans-anethole, thymol, and α-terpineol, in terms of both acute toxicity and feeding deterrence against *S. litura* (Hummelbrunner *et al.,* 2001). Citronella oil has demonstrated good efficacy against mosquitoes in concentrations ranging from 0.05% to 15.0% (w/v) alone or in combination with other natural or commercial insect repellent products (Sakulku *et al.,* 2009). *Citronella oil is used as insect repellent against mosquitoes, fleas, black flies and ticks* (Oussalah *et al.,* 2006; Koba *et al.,* 2004).

Pupal mortality was recorded by counting dead pupae which were darker in color and small in size. Pupal mortality ranged from 0.0 to 6.67% (Table 4.9). Maximum of 6.67% pupal mortality was observed in NNE-7, NNE-8, NNE-9 and NNE-10. Though, there was no statistically significant difference among them, nanoemulsions with citronella oil content more than 3.0% (NNE-7, NNE-8, NNE-9 and NNE-10) showed highest pupal mortality (6.67%) among the treatments.

Effect of neem nanoemulsions on the weight reduction of *S. litura* at 24 hrs, 48 hrs and 72 hrs intervals ranged from 49.67 to 88.0%, 50.33 to 91.0% and 52.33 to 90.33% respectively (Table 4.9). NNE-10 showed the highest larval weight reduction (91.0%) at 2.0% concentration after 48 hrs, while minimum larval weight reduction was observed in NNE-1 (49.67%) at 1.0% concentration after 24 hrs (Table 4.9). The larval weight reduction was increased when the amount of citronella oil increased from 0.5 to 5.0%. This reduction in larval weight may be due to reduced food intake and antifeedant activity of the oils.

The adult emergence was found to be between 86.67 and 0.0% in NNE-1 and NNE-10, respectively. But only 1.33% malformed adult were noticed in NNE-7, NNE-8, NNE-9 and NNE-10. In NNE-1 at 1.0 and 1.5% concentrations, 86.67% normal adult emergence occurred with 13.33% IGR activity. While using NNE-10, 100% IGR activity was observed. It was found that malformed adults were formed only at higher concentration of citronella oil (>3.00%) in nanoemulsion affected the molting and induced a wide range of anatomical abnormalities such as deformed body, incomplete wing base, twisted wing, shortened antenna, shortened mouth parts, stretched out mouth and twisted genitalia. The enhanced growth regulatory activity was due to addition effect of citronella oil in neem nanoemulsion.

Similarly, bioactivity of citronella nanoemulsions (CNEs) were studied against 3rd instar larvae of *S. litura* at three different concentrations (1.0, 1.5 and 2.0%). The mortality of larvae increased from 0.0 to 66.67%, 0.0 to 66.67% and 6.67 to 73.33% at 24 hrs, 48 hrs and 72 hrs, respectively (Table 4.10). Maximum larval mortality occurred at CNE-8, CNE-9, CNE-10 followed by CNE-7, CNE-6 and CNE-5. The remaining concentrations showed less than 50.0% mortality. These phenomena of high mortality of larvae with increase in concentration of neem oil in citronella nanoemulsion may be attributed to increased content of azadirachtin, insecticidal component of neem oil, in the nanoformulations. Citronella nanoemulsions (CNE-8,

CNE-9 and CNE-10) with 4.0% and more neem oil do not have significant difference among themselves (Table 15). These results indicated that when neem oil was added to citronella oil nanoemulsion, the insecticidal activity of the formulation was enhanced, a phenomenon which is in corroboration with the previous results (Ali *et al.,* 2017). Study showed that the nanoparticles containing azadirachtin (5000 mg/kg) were effective in combating *Plutella xylostella,* with 100% mortality of the larvae (Shah *et al*., 2016). Nanoemulsion of neem oil in Tween-20 was found as potent larvicidal agent for *Culex quinqueasciatus* (Anjali *et al.,* 2011). Okumu *et al.,* (2007) reported that the emulsions of neem oil with emulsifier and isopropanol to be effective against *Anopheles gambiae*. The compounds from neem have been reported to be effective bioinsecticides (Chary, 2011) and found useful in controlling nearly 400 insects of medical and veterinary species and flies *Amblyomma variegatum,* lice, head louse, spiders, mosquitoes cockroaches and fleas (Abdel-Ghaffar *et al*., 2012).

Pupal mortality was also recorded and found to vary from 0.0 to 6.67% (Table 4.10). The highest pupal mortality (6.67%) was observed in CNE-8, CNE-9 and CNE-10.

Also, the larval weight reduction at 24 hrs, 48 hrs and 72 hrs ranged from 49.33 to 87.67%, 49.67 to 88.33% and 50.0 to 97.67%, respectively (Table 15). CNE-10 showed highest larval weight reduction (97.67%) at 2% concentration after 72 hrs, while minimum larval weight reduction was observed in CNE-1 (49.33%) at 1.0% concentration after 24 hrs (Table 4.9). It has been found that the larval weight reduction increased when the amount of neem oil increased from 0.5 to 5.0%. This reduction in larval weight was due to reduced food intake and antifeedant activity of the oil. It was also reported that azadirachtin inhibited the production of fat body proteins resulting in weight reduction (Schluter *et al*., 1985). Similarly, neem oil was reported to cause reduction in larval weight of *Helicoverpa armigera* at 3 and 7 DAT ranging between 27.15 to 85.88% and 27.1 to 53.16%, respectively (Jaydeep, 2008).

The adult emergence was found to be between 93.33 and 13.33% in CNE-1 and CNE-10 nanoemulsions, respectively. Only 6.67% abnormal malformed adult was recorded in CNE-8, CNE-9 and CNE-10 nanoemulsions. In CNE-1 at 1.0 and 1.5% concentrations, 93.33% normal adult emergence occurred while 86.67% IGR activity was found in NNE-10. It was found that abnormal adults were formed only at higher concentration of neem oil (>4.00%) in citronella nanoemulsion. These results

are similar to previous study in which neem extracts were found to have growth regulation activity besides other activities like repellency against lice, ticks and mosquito (Tiwari *et al*., 2014). Growth regulatory and antifeedant activity of neem was due to presence of azadirachtin and other bioactive substances (Wu *et al*., 2006) and their interaction with the neuro-endocrine system of insects (Mordue *et al.*, 2005). In the present study, enhanced growth regulatory activity was due to additional effect of neem oil in nanoemulsion. Azadirachtin from the neem plant is the main component responsible for antifeedant, repellent, growth disruptant and adverse activities on reproduction against insects (Roy and Gurusubramanian, 2011). Neem extracts have shown effect on regulation of growth as well as repellency (Tiwari *et al.*, 2014). At the physiological level, azadirachtin blocks the synthesis and release of molting hormones (ecdysteroids) from the prothoracic gland leading to incomplete ecdysis in mature insects (Franck *et al.*, 2009).

6. SUMMARY AND CONCULSION

Biopesticides are considered to be safe, target specific, biodegradable and eco-friendly. These, especially *Azadirachta indica* A. Juss. (neem) based biopesticides, are mainly used as emulsified concentrate (EC). There is growing interest in nanoemulsions based on phytochemical mixtures in pest control due to their better efficacy compared to conventional biopesticides. The seed oil of *A. indica* and *Cymbopogon nardus* (L.) Rendle (Citronella) oil are known to have pest control properties. However, their utilization is often restricted due to relatively low water solubility. This drawback can be overcome by encapsulating oils in oil-in-water (O/W) emulsions or nanoemulsions using low or high energy methods. Thus the thesis entitled "**DEVELOPMENT OF INNOVATIVE FORMULATION(S) OF PHYTOCHEMICALS/OIL DERIVED FROM *AZADIRACHTA INDICA* A. JUSS AND *CYMBOPOGON NARDUS* (L.)**" had an aim to develop nanoemulsions based on neem and citronella oils for their use in pest management. The main aim of this study was to develop neem and citronella oil nanoemulsions by using low energy spontaneous emulsification methods. Another area of research was to characterize the developed nanoemulsion formulations.

Further, it was planned to study the *in vitro* bio efficacy of different developed nano formulations against phtyopathogenic fungi, *Rhizoctonia solani* and *Sclerotium rolfsii*, and against *Spodoptera litura* larvae. In this study, the nanoemulsion formulations containing neem oil, Tween 20 and deionised water (NNEs) and nanoemulsion formulations containing citronella oil, Triton-X100 and deionized water (CNEs), were successfully optimized by the low energy spontaneous emulsification method. The developed nanoemulsion formulations were characterised by Dynamic light scattering (DLS), Fourier transform infrared spectrophotometer (FT-IR), Transmission Electron Microscope (TEM), stability test and pH.

The average size of droplets of neem nanoemulsion (NNEs) with different percentage of citronella oil ranged from 11.23±3.86 nm to 17.80±4.52 nm while that of citronella nanoemulsion (CNEs) with different percentage of neem oil ranged from 8.12±2.80 nm to 12.04±3.74 nm. It was found that increase in surfactant ratio to neem oil or citronella oil decreases the size of droplets in nanoemulsions. The smallest

droplet size of 11.23±2.78 nm for neem nanoemulsions (NNEs) and 8.12±2.80 nm for citronella nanoemulsions (CNEs) were obtained.

The loading of citronella and neem oil in the nanomicelle was confirmed using FT-IR, which showed characteristic bands of both the oils in the nanomicelle. TEM study showed the spherical shape of neem and citronella oil nanoemulsions.

The developed nanoemulsion formulations were stable as per CIPAC guidelines. No foaming and separation of oily matter was seen even after 24 hrs. The nanoemulsions formed stable nearly transparent or translucent colloidal system on dilution with standard hard water D. The emulsion stability on standing was checked and found that no free oil, frothing or any cream was formed in the test cylinders. Again on re-emulsification, no abnormality and separation of cream, froth and oil was observed.

Further, *in vitro* bioefficay evaluation of developed nano formulations was done against *R. solani* and *S. rolfsii* by poisoned food technique method. Results showed that neem nanoemulsion 10 (NNE-10) and citronella nanoemulsion 10 (CNE-10) were most active against *R. solani* (ED_{50} 13.67 mg L^{-1} and 25.64 mg L^{-1}) and *S. rolfsii* (ED_{50} 14.71 mg L^{-1} and 20.88 mg L^{-1}) respectively. The standardization of the composition and development of citronella essential oil and neem oil based nanoemulsions and their antifungal activity against these two fungi has been reported for the first time.

Also developed nanoemulsion formulations were evaluated for their larvicidal and growth regulatory activity against 3rd instar larvae of *S. litura* (Fab) in laboratory conditions. In case of neen nanoemulsions (NNEs), it was found that increased concentration of citronella oil in the neem nanoemulsion resulted in enhanced larval mortality.

The mortality of larvae increased from 0.00 to 80.00%, 0.00 to 60.00% and 13.33 to 80.00% at 24 hrs, 48 hrs and 72 hrs respectively. Maximum larval mortality was observed in NNE-8 (citronella oil 4%), NNE-9 (citronella oil 4.5%) and NNE-10 (citronella oil 5%) (80.00%) after 72 hrs followed by NNE-6 (66.67%) and NNE-5 (60.00%) as compared to control (0.00%) whereas, in all remaining concentrations, the mortality was less than 50.00%.

Pupal mortality ranged from 0.00 to 6.67% (Table 14). Nanoemulsions with citronella oil content more than 3.00% (NNE-7, NNE-8, NNE-9 and NNE-10) showed highest pupal mortality (6.67%).

The mean per cent larval weight reduction at 24 hrs, 48 hrs and 72 hrs ranged from 49.67 to 88.00%, 50.33 to 91.00% and 52.33 to 90.33% respectively (Table 14). NNE-10 showed the highest larval weight reduction (91.00%) at 2.00% concentration after 48 hrs, while minimum larval weight reduction was observed in NNE-1 (49.67%) at 1.00% concentration after 24 hrs (Table 14). The larval weight reduction increased when the amount of citronella oil increased from 0.50 to 5.00%.

The adult emergence was found to be between 86.67 and 0.00% at NNE-1 and NNE-10, respectively as against 86.67% in control. But only 1.33% abnormal malformed adult were noticed at NNE-7, NNE-8, NNE-9 and NNE-10. At NNE-1 (1 and 1.5%) concentrations, 86.67% normal adult emergence occurred with 13.33% IGR activity. While using NNE-10, 100% IGR activity was observed. It was found that abnormal adults were formed only at higher concentration of citronella oil (>3.00%) in nanoemulsion. The enhanced growth regulatory activity of *S. litura* was due to addition effect of citronella oil in neem nanoemulsion.

Bioactivity of citronella nanoemulsions (CNEs) was studied against 3rd instar larvae of *S. litura*. The mortality of larvae increased from 0.00 to 66.67%, 0.00 to 66.67% and 6.67 to 73.33% at 24 hrs, 48 hrs and 72 hrs respectively. Maximum larval mortality was observed in CNE-8 (citronella oil 4.00%), CNE-9 (citronella oil 4.50%), and CNE-10 (citronella oil 5%) (73.33%) after 72 hrs followed by CNE-7, CNE-6 (60.00%) and CNE-5 (53.33%) whereas, in all remaining concentrations the mortality was less than 50.00%. These phenomena of high mortality of larvae with increase in concentration of neem oil in citronella nanoemulsion may be attributed to increased content of azadirachtin, insecticidal component of neem oil, in the nano formulation (Kumar and Parmar, 1996). Citronella nanoemulsions (CNE-8, CNE-9 and CNE-10) with 4.00% and more neem oil do not have significant difference among themselves (Table 15). These results indicate that when neem oil is added to the citronella oil nanoemulsion, the insecticidal activity of the formulation was enhanced,

Pupal mortality ranged from 0.00 to 6.67% (Table 15). A maximum of 6.67% pupal mortality was observed in CNE-8, CNE-9 and CNE-10. Though there was no

statistically significant difference among them, nanoemulsions with neem oil content more 4.00% (CNE-8, CNE-9 and CNE-10) showed highest pupal mortality (6.67%).

The mean% larval weight reduction at 24 hrs, 48 hrs and 72 hrs ranged from 49.33 to 87.67%, 49.67 to 88.33% and 50.00 to 97.67% respectively (Table 15). CNE-10 showed the highest larval weight reduction (97.67%) at 2% concentration after 72 hrs, while minimum larval weight reduction was observed in CNE-1 (49.33%) at 1.00% concentration after 24 hrs (Table 15). The larval weight reduction was increased when the amount of neem oil increased from 0.50 to 5.00%. The adult emergence was found to be between 93.33 and 13.33% in CNE-1 and CNE-10, respectively as against 86.67% in control. But only 6.67% malformed adults were noticed at CNE-8, CNE-9 and CNE-10. At CNE-1 (1 and 1.5%) concentrations, 93.33% normal adult emergence occurred with 6.67% IGR activity. While using NNE-10, 86.67% IGR activity was observed. It was found that abnormal adults were formed only at higher concentration of neem oil (>4.00%) in citronella nanoemulsion.

In the present study, neem and citronella nanoemulsions were found to be effective in controlling phytopathogenic fungi *R. solani*, *S. rolfsii* and the larvae of *S. litura*. The reduced size increased the fungcidal and larvicidal efficacy. Developed nanoemulsions, based on neem and citronella oil, can be utilized as an alternative for the management of pests as these can be easily prepared, are economically viable and less toxic to the environment than synthetic pesticides.

Development of innovative formulation (s) of phytochemicals/oil derived from *Azadirachta indica* A. Juss and *Cymbopogon nardus* (L.)

ABSTRACT

Biopesticides are safe, target specific, biodegradable and eco-friendly. *Azadirachta indica* seed oil and *Cymbopogon nardus* (L.) Rendle (Citronella) oil are known to have pesticidal properties. However, their utilization is often restricted due to relatively low water solubility. This drawback can be reduced by encapsulating oils in oil-in-water (O/W) emulsions or nanoemulsions using low or high energy methods. In this study, various nanoemulsions of crude neem and citronella oils with surfactants were developed. The physio-chemicals properties of developed formulations (Particles size, TEM analysis, FT-IR, stability test and pH) were determined. The average size of droplets of neem nanoemulsions (NNEs) with different percentage of citronella oil ranged from 11.23±3.86 nm to 17.80±4.52 nm while that of citronella nanoemulsions (CNEs) with different percentage of neem oil ranged from 8.12±2.80 nm to 12.04±3.74 nm. It was found that increased surfactant ratio to neem or citronella oil decreases the size in nanoemulsions. The standardization of the composition and development of citronella essential oil and neem oil based nanoemulsions and their antifungal activity against these two fungi has been reported for the first time. NNE-10 and CNE-10 were most active against *R. solani* (ED_{50} 13.67 mg L^{-1} and 25.64 mg L^{-1}) and *S. rolfsii* (ED_{50} 14.71 mg L^{-1} and 20.88 mg L^{-1}) respectively. The neem and citronella nanoemulsion formulations have been evaluated for their larvicidal and growth regulatory activity against 3rd instar of *Spodoptera litura* (Fab) in laboratory conditions. Maximum (80.0%) larval mortality against 3rd instar larvae of *S. litura* occurred in NNE-7, NNE-8, NNE-9 and NNE-10 after 72 hrs, while maximum (73.33%) larval mortality occurred in CNE-8, CNE-9 and CNE-10 after 72 hrs. NNE-10 and CNE-10 showed the highest larval weight reduction (91.0% and 97.67%) at 2.0% concentration after 48 and 72 hrs respectively. Developed nanoemulsions, based on neem and citronella oil, can be utilized as an alternative for the management of pests as these can be easily prepared and are economically viable and less toxic to the environment than synthetic pesticides.

एज़ाडायरेक्टा इंडिका ए. जस (एवं) सिम्बोपोगोन नार्डस(एल.) से व्युत्पन्न पादप रसायनों/तेल की नवोन्मेषी फार्मूलेशन (फार्मलूशनों) का विकास

सार

जैव–नाशीजीवनाशी सुरक्षित,लक्ष्य विशिष्ट, जैव–निम्नीकरण योग्य एंव पर्यावरण–अनुकूल हैं।एज़ाडायरेक्टा इंडिका के बीजों के तेल और सिम्बोपोगोन नार्डस (एल.) रेंडल (सिट्रोनैला) तेल में नाशीजीवनाशक गुण विद्यमान होते हैं। तथापि,आपेक्षिक रूप से कम जल घुलनशीलता के कारण उनका उपयोग प्रायः सीमित है। न्यून एंव उच्च ऊर्जा विधियों का उपयोग कर, जल–में–तेल (ओ/डब्लू) इमल्शन या नैनोइमल्शन में तेलों को कैप्स्यूल में बंद कर इस समस्या को कम किया जा सकता है। इस अध्ययन में, अपरिष्कृत नीम एंव सिट्रोनैला तेल को पृष्ठ–सक्रियकों के साथ मिलाकर कई नैनोइमलशन विकसित किए गए हैं। विकसित की गई इन फार्मूलेशनों के भौतिक–रासायनिक गुण (कण–परिमाण, टी ई एम विश्लेषण,एफ टी आई आर, स्थायित्व एंव पी एच मान) ज्ञात किए गए। सिट्रोनैला तेल की भिन्न–भिन्न प्रतिशतता के साथ नीम नैनोइमल्शन (एन एन ई) की बूंदों का औसत परिमाण 11.23 + 3.86 नैनो मीटर से 17.80 + 4.52 नैनोमीटर की सीमा में था जबकि नीम के तेल की भिन्न प्रतिशतता के साथ सिट्रोनैला नैनोइमल्शन (सी एन ई) की बूंदों का औसत परिमाण 8.12 +2.80 नैनो मीटर से 12.04 + 3.74 नैनोमीटर की सीमा में था। यह पाया गया कि नीम या सिट्रोनैला तेल में पृष्ठ–सक्रियक का अनुपात बढ़ाने से नैनोइमल्शन का परिमाण कम हो जाता हैं। सिट्रोनैला आवश्यक तेल और नीम तेल आधारित नैनोफामूलेशनों का विकास एवं उनकी संरचना का मानकीकरण तथा दो कवकों के विरूद्ध इनकी कवकविरोधी सक्रियता के विषय में यह प्रथम सूचना है। एन एन ई–10 क्रमशः आर. सोलेनाई(ई डी50 13.67 मिग्रा प्रति ली एवं 25.64 मिग्रा प्रति ली)एवं एस.रोल्फसाई(ई डी50 14.71 मिग्रा प्रति ली एवं 20.88 मिग्रा प्रति ली) के विरूद्ध सर्वाधिक सक्रिय पाए गए। प्रयोगशाला परिस्थितियों के अंतर्गत, स्पोडोप्टोरा लिट्यूरा (फैब) के तीसरे इन्सटार के विरूद्ध नीम एवं सिट्रोनैला नैनोइमल्शन फार्मूलेशन का उनकी लार्वानाशी एवं वृद्धि नियामक सक्रियता हेतू मूल्यांकन किया गया। एन एन ई–7, एन एन ई–8, एन एन ई–10 में 72 घंटे पश्चात एस.लिट्यूरा के तीसरे इन्सटार लार्वों के लिए अधिकतम(80.0:) लार्वा–मर्त्यता देखी गई जबकि सी एन ई–8, सी एन ई–9 एवं सी एन ई–10 में 72 घंटे पश्चात अधिकतम लार्वा–मर्त्यता देखी गई। एन एन ई–10 एवं सी एन ई–10 ने 2.0:सोद्रता पर क्रमशः 48 एवं 72 घंटे पश्चात, लार्वा मे भार की अधिकतम कमी (91.0:) एवं 97–67:) दर्शायी । नीम एवं सिट्रोनैला के तेल पर आधारित, विकसित की गई इन फार्मूलेशनों का नाशीजीवों के प्रबंधनार्थ एक विकल्प के रूप में उपयोग किया जा सकता है क्योंकि इन्हें तैयार करना सरल है, आर्थिक रूप से उपयोगी हैं और संश्लेषित नाशीजीवनाशियों की तुलना में पर्यावरण के लिए इनकी विषाक्तता नगण्य है।

BIBLIOGRAPHY

Abbas, N., Samiullah., Sdad, S. A., Razag, M., Waheed, A., Aslam, M. (2014) Resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to profenofos: Relative fitness and cross resistance. *Crop Prot.* **58:** 49-54.

Abbas, N., Shad, S. A., Razaq, M. (2012) Fitness cost, cross resistance and realized heritability of resistance to imidacloprid in *Spodoptera litura* (Lepidoptera: Noctuidae). *Pestic. Biochem. Physiol*. **103 (**3**)**: 181-188.

Abdel-Ghaffar, F., Al-Quraishy, S., Al-Rasheid, K. A. S., Mehlhorn, H. (2012) Efficacy of a single treatment of head lice with a neem seed extract: an *in vivo* and *in vitro* study on nits and motile stages. *Parasitol. Res.* **110** (1): 277-280.

Abdel-Ghaffar, F., Semmler, M. (2007) Efficacy of neem seed extracts shampoo on head lice of naturally infected humans in Egypt. *Parasitol. Res*. **100** (2): 329-332.

Abdul Razak, T., Santhakumar, T., Mageswari, K., Santhi, S. (2014) Studies on efficacy of certain neem products against *Spodoptera litura* (Fab.*). J. Biopest.* **7** (supp): 160-163.

Adeyemi, M. M. H. (2010) The potential of secondary metabolites in plant material as deterents against insect pests: A review. *Afr. J. Pure Appl. Chem.* **4** (11): 243-246.

Agarwal, K. K. (2008) Chemical composition of major essential oils of India. Swarat Herbal Plants, Barabanki, UP, India.

Aggarwal, N., Kumar, R., Dureja, P., Rawat, D. S. (2009) Schiff basses as potential fungicides and nitrification inhibitors. *J. Agric. Food Chem*. **57** (18): 8520-8525.

Agrawal, N., Maddikeri, G. L., Pandit, A. B. (2017) Sustained release formulations of citronella oil nanoemulsion using cavitational techniques. *Ultrason. Sonochem*. **36**: 367-374.

Agrios, G. N. (2015) Plant Pathology, Sixth edition. Academic Press, New York.

Ahmad, M., Arif, M. I., Ahmad, M. (2007) Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *Crop Prot*. **26** (6): 809-817.

Ahmad, M., Sayyed, A. H., Saleem, M. A., Ahmad, M. (2008) Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. *Crop Prot*. **27** (10): 1367-1372.

Akhila, A., Rani, K., (1999) Chemistry of the neem tree (*Azadirachta indica* A. Juss.). *Fortschr.Chem. Org.* **78:** 47-149.

Akin-Osanaiye, B. C., Nok, A. J., Ibrahim, S., Inuwa, H. M., Onyike, E., Amlabu, E., Haruna, E. (2013) Antimalarial effect of neem leaf and neem stem bark extracts on plasmodium berghei infected in the pathology and treatment of malaria. *I. J. B. B.* **3** (1): 7-14.

Ali, E. O. M., Shakil, N. A., Rana, V. S., Srivastava, C., Sarkar, D. J., Parshant, K. (2017) Insecticidal effect of citronella and neem oil against *Spodoptera litura* (Fab). *Ann. Pl. Protect. Sci.* **25** (2): 231-238.

Ali, M., Singh, R., Lal, M., Ali, I., Zuhaib, M., Kumar, S. (2015) Evaluation of different plant based essential oil against Rhizoctonia causing sheath blight of rice. *Agriways.* **3** (1): 10-13.

Almeida, R. N. D., Agra, M. F., Maior, F. N. S., Sousa, D. P. D. (2011) Essential Oils and Their Constituents: Anticonvulsant Activity. *Molecules* **16** (3): 2726-2742.

Anjali, C. H., Sharma, Y., Mukherjee, A., Chandrasekaran, N. (2011) Neem oil (*Azadricta indica*) nanoemulsion a potent larvicidal agent against (*Culex quinquefasciats*. *Pest Manage. Sci.* **68** (2): 158-163.

Anton, N., Vandamme, T., F. (2011) Nanoemulsions and microemulsions: Clarifications of the critical differences. *Pharm. Res.* **28** (5): 978-985.

Anyaehie, U. B. (2009) Medicinal properties of fractionated acetone/water neem (*Azadirachta indica*) leaf extract from Nigeria: a review. *Niger J. Physiol. Sci*. **24** (2): 157-159.

Baboota, S., Shakeel, F., Ahuja, A., Ali, J., Shafiq, S. (2007) Design, development and evaluation of novel nanoemulsions for transdermal potential of celecoxib. *Acta Pharm*. **57** (3): 315-332.

Benelli, G. (2015) The best time to have sex: mating behaviour and effect of daylight time on male sexual competitiveness in the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae). *Parasitol. Res.* **114** (3): 887-894.

Bhagat, D., Samanta, S. K., Bhattacharya, S. (2013) Efficient management of fruit pests by pheromone nanogels. *Sci. Rep.* **3** (1294): 1-8.

Bin Hussein, M. Z., Zainal, Z., Yahaya, A. H., Foo, D. W. V. (2002) Controlled release of a plant growth regulator, alpha-naphthaleneacetate from the lamella of Zn-Al-layered double hydroxide nanocomposite. *J. Control Release*. **82** (2-3): 417-427.

Biswas, K., Chattopadhyay, I., Ranajit, K. B., Bandyopadhyay, U. (2002) Biological activities and medicinal properties of neem (*Azadirachta indica*). *Curr. Sci.* **82** (11): 1336-1345.

Boehm, A. L. L., Martinon, I., Zerrouk, R., Rump, E., Fessi, H. (2003) Nanoprecipitation technique for the encapsulation of agrochemical active ingredients. *J. Microencapsul.* **20** (4): 433-441.

Boeke, S. J., Boersma, M. G., Alink, G. M., Van Loon, J. J. A., Van Huis, A., Dicke, M., Rietjens, I. M. (2004) Safety evaluation of neem (*Azadirachta indica*) derived pesticides. *J. Ethnopharmacol*. **94** (1): 25-41.

Bokhari, N. A., Siddiqui, I., Perveen, K., Siddique, I., Soliman, D. W. A. (2015) Mycocidal ability of ciliata against *Rhizoctonia solani. J. Anim. Plant Sci.* **25** (5): 1477-1481.

Botelho, M. A., Dos, S. R. A., Martins, J. G., Carvalho, C. O., Paz, M. C., Azenha, C., Ruela, R. S., Queiroz, D. B., Ruela, W. S., Marinho, G., Ruela, F. I. (2008) Efficacy of a mouth rinse based on leaves of the neem tree (*Azadirachta indica*) in the treatment of patients with chronic gingivitis: A double blind, randomized, controlled trial. *J. Med. Plant Res*. **2** (11): 341-346.

Brunel, F, El Gueddari, N. E., Moerschbacher, B. M. (2013) Complexation of copper (II) with chitosan nanogels: toward control of microbial growth. *Carbohydr. Polym*. **92** (2): 1348-1356.

Cao, Y., Chen, J., Wang, Y., Liang, J., Chen, L. H., Lu, Y. (2005) HPLC/UV analysis of chlorfenapyr residues in cabbage and soil to study the dynamics of different formulations. *Sci. Total Environ*. **350** (1-3): 38-46.

Chang, Y., Mclandsborough, L., McClements, D. J. (2012) Physical properties and antimicrobial efficacy of thyme oil nanoemulsions: influence of ripening inhibitors. *J. Agric. Food Chem.* **60** (48): 12056-12063.

Chang, Y., McLandsborough, L., McClements, D. J. (2013) Physicochemical properties and antimicrobial efficacy of carvacrol nanoemulsions formed by spontaneous emulsification. *J. Agric. Food Chem*. **61** (37): 8906-8913.

Chary, P (2011) A comprehensive study on characterization of elite Neem chemotypes through mycofloral, tissue-cultural, ecomorphological and molecular analyses using azadirachtin-A as a biomarker. *Physiol. Biol. Plants*. **17** (1): 49-64.

Chatterjee, R., Singh, O., Pachuau, L., Malik, S. P., Paul, M., Bhadra, K., Paul, S., Kumar, G. S., Mondal, N. B., Banerjee, S. (2010) Identification of a sulfonoquinovosyldiacylglyceride from *Azadirachta indica* and studies on its cytotoxic activity and DNA binding properties. *Bioorganic Med. Chem. Lett*. **20** (22): 6699-6702.

Choochote, W., Chaithong, U., Kamsuk, K., Jitpakdi, A., Tippawangkosol, P., Tuetun, B., Champakaew, D., Pitasawat, B. (2007) Repellent activity of selected essential oils against *Aedes aegypti*. *Fitoterapia*. **78** (5): 359-364.

CIPAC Hand book (1970) Analysis of technical and formulated pesticides, Compiled by Ashworth, R., Henriet, J., Lovett, J. F., Ed. G. R. Raw., Collaborative International Pesticides Council Ltd. 1079 pages.

Costa, J. T., Forim, M. R., Costa, E. S., Souza, J. R. D., J. M., Juniora, A. L. B. (2014) Effects of different formulations of neem oil-based products on control *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Bruchidae) on beans. *J. Stored Prod. Res*. **56**: 49-53.

Darshan, S., Doreswamy, R. (2004) Patented anti-inflammatory plant drug development from traditional medicine. *Phytother. Res*. **18** (5): 343-357.

De Mello, V., Prata, M. C., Da Silva, M. R., Daemon, E., Da Silva, L. S., Fdel, G. G., De Mendonça, A. E., Folly, E., Vilela, F. M., Do Amaral, L. H., Cabral, L. M., Mda, D. P. (2014) Acaricidal properties of the formulations based on

essential oils from *Cymbopogon winterianus* and *Syzygium aromaticum* plants. *Parasitol. Res*. **113** (12): 4431-4437.

Devakumar, C., Kumar, R. (2007) Agro-biochemicals in agriculture. In ICAR-Industry Meet-Agricultural transformation through public private partnership: an interface; Ayyappan, S., Chandra, P., Tandon, S. K., eds., Directorate of Information and Publication of Agriculture, ICAR: New Delhi, India, 55-62.

Dhaliwal, G. S., Arora, R. (2001) Integrated pest management. Kalyani Publishers, New Delhi, P427.

Dhayanithi, N. B. 1., Kumar, T. T., Kathiresan. K. (2010) Effect of neem extract against the bacteria isolated from marine fish. *J. Environ. Biol*. **31** (4): 409-412.

Dissanayake, M. L. M. C., Jayasinghe, J. A. N. (2013) Antifungal activity of selected medicinal plant extracts against plant pathogenic fungi; *Rhizoctonia solani*, *Colletotrichum musea* and *Fusarium oxysporum*. *Int. J. Sci. Inven. Today*. **2** (5): 421-431.

Dixit, V. P., Sinha, R., Tank, R. (1986) Effect of neem seed oil on the blood glucose concentaration of normal and alloxan diabetic rats. *J. Ethnopharmacol*. **17** (1): 95-8.

Dubey, N. K., Shukla, R., Kumar, A., Singh, P., Prakash, B. (2010) Prospects of botanical pesticides in sustainable agriculture. *Curr. Sci*. **98** (4): 25.

Egho, E. O., Ilondu, E. M. (2012) Seeds of neem tree (*Azadirachta indica* A. Juss). Promising biopesticide in the management of cowpea insect pests and grain yield in the early cropping season at Asaba and Abraka, Delta State, Nigeria. *J. Agric. Sci.* **4** (1): 181-189.

Elavarasu, S., Abinaya, P., Elanchezhiyan, S., Vennila, T. K., Naziya, K. B. (2012) Evaluation of anti-plaque microbial activity of *Azadirachta indica* (neem oil) *in vitro*: A pilot study. *J. Pharm. Bioall. Sci*. **4** (2): 394-396.

El-Nahhal, Y., Nira, S., Margulies, L., Rubin, B. (1999) Reduction of photo degradation and volatilization of herbicides in organo-clay formulations. *App. Clay Sci*. **14** (1-3): 105-119.

Errakhi, R., Bouteau, F., Lebrihi, A., Mustapha, B. (2007) Evidences of biological capacities of *Streptomyces* spp against *Sclerotium rolfssi* responsible for

damping-off disease in sugar beet (*Beta vulgaris* L.). *World J. Microbiol.* **23**: 1503-1509.

Forgiarini, A., Esquena, J., Gonza, C., Solans, C. (2001) Formation of nanoemulsions by low-energy emulsification methods at constant temperature. *Langmuir.* **17** (7): 10762-083.

Fradin, M. S. (1998) Mosquitoes and mosquito repellents: a clinician's guide. *Ann. Intern. Med.* **128** (11): 1931-1940

Franck, E. D., Charles, L. C., Stephen. O. D. (2009) Natural products in crop protection. *Bioorganic Med. Chem.* **17**: 4022-4034.

Gahukar, R. T. (2014). In: Abrol, D. P. (Ed.), Integrated Pest Management: Current Concepts and Ecological Perspective. Elsevier Inc, New York, NY, USA, pp. 125-139.

Galhardi, L. C., Yamamoto, K. A., Ray, S., Ray, B., Linhares, R. E. C., Nozawa, C. (2012) *In vitro* antiviral property of *Azadirachta indica* polysaccharides for poliovirus. *J. Ethnopharmacol.* **142** (1): 8690.

Gatta, M. A., Ippolito, A., Linsalataa, V., Cascarano, N. A., Nigro, F., Vanadia, S., Venere, D. D. (2011) Activity of extracts from wild edible herbs against postharvest fungal diseases of fruit and vegetables. Postharvest. *Biol. Technol.* **61** (1): 72-82.

Gavrilescu, M. (2005) Fate of Pesticides in the environment and its bioremediation. *Eng. Life Sci*. **5** (6): 497-526.

Ghorbanian, M., Razzaghi-Abyaneh, M., Allameh, A., Shams-Ghafarokhi, M., Qorbani, M. (2008) Study on the effect of Neem *(Azadirachta indica* A. Juss) leaf extract on the growth of *Aspergillus parasiticus* and production of aflatoxin by it at different incubation times. *Mycoses.* **51** (1): 35-39.

Ghosh, A., Chowdhury, N., Chandra, G. C. (2012) Plant extracts as potential mosquito larvicides. *Indian J. Med. Res*. **135** (5): 581-598.

Ghotbi, R. S., Khatibzadeh, M., Kordbacheh, S. (2014) Preparation of neem seed oil nanoemulsion. *Proceedings of the 5th International conference on nanotechnology: Fundamentals and applications*, 150-155.

Guan, H. A., Chi, D. F., Yu, J., Li, H. (2010) Dynamics of residues from a novel nanoimidacloprid formulation in soybean fields. *J. Crop Prot*. **29** (9): 942-946.

Guan, H., Chi, D. F., Yu, J. C., Li, X. (2008) A novel photodegradable insecticide: Preparation, characterization and properties evaluation of nanoimidacloprid. *Pestic. Biochem. Physiol.* **92** (2): 83-91.

Hubbe, M. A., Rojas, O. J., Lucia, L. A., Sain, M. (2008) Cellulosic nanocomposites: A review. *Bioresources.* **3** (3): 929-980.

Hummelbrunner, L. A., Isman, M. B. (2001) Acute, sub lethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). *J. Agric. Food Chem.* **49** (2): 715-720.

Hwang, Y. Y., Ramalingam, K., Bienek, D. R., Lee, V., You, T., Alvarez, R. (2013) Antimicrobial activity of nanoemulsion in combination with Cetylpyridinium chloride in multidrug-resistant *Acinetobacter baumannii*. *Antimicrob. Agents Chemother*. **57** (8): 3568-3575.

Isman, M. B. (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol*. **51**:45-66.

Iyer, S. R., Williamson, D. G. (1991) Studies on the occurrence of keratinophilic fungi in the soil of Rajasthan-II. *Indian Phytopathol*. **44** (4): 487-490.

Jaenson, T. G., Garboui, S., Palsson, K. (2006) Repellency of oils of lemon eucalyptus, geranium, and lavender and the mosquito repellent MyggA natural to Ixodes ricinus (Acari: Ixodidae) in the laboratory and field. *J. Med. Entomol.* **43** (4): 731-6.

Jaydeep, H. (2008) Studies on the potential of plant extracts and their mixtures for the management of *Helicoverpa armigera*, PhD thesis, Division of Entomology, Indian Agricultural Research Institute, New Delhi, India.

Jerobin, J., Sureshkumar, R. S., Anjali, C. H., Mukherjee, A., Chandrasekaran, N., (2012) Biodegradable polymer based encapsulation of neem oil nanoemulsion for controlled release of Aza-A. *Carbohydr. Polym*. **90** (4): 1750-1756.

Kagale, S., Marimuthu, T., Thayumanavan, B., Nandakumar, R., Samiyappan, R. (2004) Antimicrobial activity and induction of systemic resistance in rice

by leaf extract of Datura metel against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. Oryzae. *Physiol. Mol. Plant Path*. **65** (2): 91-100.

Kah, M., Beulke, S., Tiede, K., Hofmann, T. (2013) Nanopesticides: State of Knowledge, Environmental Fate, and Exposure Modeling. *Crit. Rev. Environ. Sci. Technol*. **43** (16): 1823-1867.

Karthik, P., Ezhilarasi, P. N., Anandharamakrishnan, C. (2017) Challenges associated in stability of food grade nanoemulsions. *Crit. Rev. Food Sci. Nutr*. **57** (7): 1435-1450.

Karunamoorthi, K., A., Mulelam, A., Wassie, F. (2009) Assessment of knowledge and usage custom of traditional insect/ mosquito repellent plants in addis Zemen Town, South Gonder, Notrh estern Ethiopia. *J. Ethnopharmacol*. **121**: 49-53.

Katagi, T. (2008) Surfactant effects on environmental behavior of pesticides. *Rev. Environ. Contam. Toxicol.* **194**: 71-177.

Kaushik**,** P., Shakil, N. A., Kumar, J., Singh, M. K., Yadav, S. K. (2013) Development of controlled release formulations of thiram employing mphiphilic polymers and their bioefficacy evaluation in seed quality enhancement studies. *J. Environ. Sci. Health B.* **48** (8): 677-685.

Khalil, M. S. (2013) Abamectin and Azadirachtin as eco-friendly promising biorational tools in integrated nematodes management programs *J. Plant Pathol. Microbiol.* **4** (3): 1-7.

Khan, M., Wassilew, S. W. (1987) Natural Pesticides from the Neem Tree and Other Tropical Plants (eds) Schmutterer, H., Asher, K. R. S., GTZ: Eschborn, Germany, 1987, pp. 645.

Khater, H. (2011) Ecosmart biorational insecticides: alternative insect control strategies. *Adv. Integr. Pest Manag. InTech. Croat*. 17-60.

Klocke, J. A., 1 (989) Plant compounds as source and models of insect control agents. In: Hostettmann, K (ed) Economic and medicinal plant research. Academic, London, pp 103–144.

Knowles, A. (2005) New developments in crop protection product formulation. *Agrow Report.* UK, pp 153-156.

Koba, K., Sanda, K., Raynaud, C., Nenonene, Y. A., Millet, J., Chaumont, J. P. (2004) "Activités anti microbiennesd'huilesessentielles d' trios Cymbopogon sp. Africains vis- avis de germespathogènesd'animaux de compagnie."*Annales de MédecineVétérinaire,* **148**: 202-206.

Koli, P., Singh, B. B., Shakil, N. A., Kumar, J., Kamil, D. (2014) Development of controlled release nanoformulations of carbendazim employing amphiphilic polymers and their bioefficacy evaluation against *Rhizoctonia solani*. *J. Environ. Sci. Health, Part B.* **50** (9): 674-681.

Kookana, R. S., Boxall, A. B. A., Reeves, P. T., Ashauer, R., Beulke, S., Chaudhry, Q., Cornelis, G., Fernandes, T. F., Gan, J., Kah, M., lynch, I., Ranville, J., Sinclair, C., Spurgeon, D., Tiede, K., Brink, P. J. V. D. (2014) Nanopesticides: guiding principles for regulatory evaluation of environmental risks. *J. Agric. Food Chem*. **62** (19): 4227-4240.

Koul, O., Walia, S., Dhaliwal, G. S. (2008) Essential Oils as Green Pesticides: Potential and Constraints. *Biopestic. Int.* **4** (1): 63-84.

Kranthi, K. R., Jadhav, D. R., Kranthi, S., Wanjari, R. R., Ali, S. S., Russell, D. A. (2002) Insecticide resistance in five major insect pests of cotton in India. *Crop Prot.* **21** (6): 449-460.

Kumar, J., Verma, V., Goyal, A., Shahi, A. K., Sparoo, R., Sangwan, R. S., Qazi, G. N. (2009) Genetic diversity analysis in Cymbopogon species using DNA markers. *Plant Omic. J.* 2 (1): 20-29.

Kumar, S., Srivastava, K. (2013) Screening of tomato genotypes against early blight (*Alternaria solani*) under field condition. *The Bioscan*. **8** (1): 189-193.

Kumar, V. S., Navaratnam, V. (2013) Neem (*Azadirachta indica*): Prehistory to contemporary medicinal uses to humankind. *Asian Pac. J. Trop. Biomed*. **3** (7): 505-514.

Labinas, A. M., Crocomo, W. B. (2002) Effect of java grass (*Cymbopogon winterianus*) essential oil on fall army worm *Spodoptera frugiperda* (J. E. Smith, 1979) (Lepidoptera, Noctuidae). *Acta Sci.* **24** (5): 1401-1405.

Lai, F., Wissing, S. A., Müller, R. H. Fadda, A. M. (2006) *Artemisia arborescens* L essential oil-loaded solid lipid nanoparticles for potential agricultural application: Preparation and characterization. *Pharm. Sci. Tech.* **7** (1): E10.

Lawrence, M. J., Rees, G. D. (2000) Microemulsion-based media as novel drug delivery systems. *Adv. Drug Deliv. Rev*. **45** (1): 89-121.

Li, W. R., Shi, Q. S., Ouyang, Y. S., Chen, Y. B, Duan, S. S. (2013) Antifungal effects of citronella oil against *Aspergillus niger* ATCC 16404. *Appl. Microbiol. Biotechnol.* **97** (16): 7483-7492.

Liu, F., Wen, L. X., Li, Z. Z., Yu, W., Sun, H. Y., Chen, J. F. (2006) Porous hollow silica nanoparticles as controlled delivery system for water-soluble pesticide. *Mater. Res. bull.* **41** (12): 2268-2275.

Lucantoni, L., Giusti, F., Cristofaro, M., Pasqualini, L., Esposito, F., Lupetti, P., Habluetzel, A. (2006) Effects of neem extract on blood feeding oviposition and oocyte ultra structure in *Anopheles stephensi* Liston (Diptera: Culicidae). *Tissue Cell*. **38** (6): 361-371.

Luong, K., Dunkel, F. V., Coulibaly, K., Beckage, N. E. (2012) Potential use of neem leaf slurry as a sustainable dry season management strategy to control the malaria vector *Anopheles gambiae* (Diptera: Culicidae) in west African villages. *J. Med. Entomol*. **49** (6): 1361-1369.

Mahapatra, S., Karnes. R. J., Holmes, Michael, W., Young, C. Y. F., Cheville, J. C, Kohli, M., Klee, E. W., Tindall, D. J., Donkena, K. V. (2011) Novel molecular targets of *Azadirachta indica* associated with inhibition of tumor growth in prostate cancer. *AAPS J*. **13** (3): 365-377.

Maia, A. J., Oliveira, J. S. B., Schwan-Estrada, K. R. F., Faria, C. M. R., Batista, A. F., Costa, W. F., Batista, B. N. (2014) The control of isariopsis leaf spot and downy mildew in grapevine cv. Isabel with the essential oil of lemon grass and the activity of defensive enzymes in response to the essential oil. *Crop Prot.* **63**: 57-67.

Maity, P., Biswas, K., Chattopadhyay, I., Banerjee, R. K., Bandyopadhyay, U. (2009) The use of neem for controlling gastric hyperacidity and ulcer. *Phytother res*. **23** (6): 747-755.

Majumder, S., Shakil, N. A., Singh, B. B., Waterson, A. C. (2016) Synthesis and characterization of functionalized amphiphilic polymers for utilization as surfactants. *J. Macromol. Sci. Part A: Pure. Appl. Chem*. **53** (2): 1-7.

Mao, L., Xu, D., Yang, J., Yuan, F, Gao, Y., Zhao, J. (2009) Effects of small and large molecule emulsifiers on the characteristics of β-carotene

nanoemulsions prepared by high pressure homogenization. *Food Technol. Biotechnol.* **47** (3): 336-342.

Maqsood, S., Sabri, M. A., Ali, A., Abbas, M., Aziz, A. (2016) Comparative toxicity of some insecticides against army worm, L. (Lepidoptera: Noctuidae) under laboratory conditions. *J. Entomol. Zool. Stud.* **5** (1): 770-773.

Maqueda, C., Villaverde, J., Sopena, F., Undabeytia, T., Morillo, E. (2009) Effects of soil characteristics onmetribuzin dissipation using clay-gel-based formulations. *J. Agric. Food Chem*. **57** (8): 3273-3278.

McClements, D. J., Rao, J. (2011) Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Crit. Rev. Food Sci. Nutr.* **51** (4): 285−330.

Mei, Z., Chen, H., Weng, T., Yang, Y., Yang, X. (2003) Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. *Eur. J. Pharm. Biopharm*. **56** (2): 189-196.

Mew, T. W., Cottyn, B., Pamplona, R., Barrios, H., Xiangmin, L., Zhiyi, C., Fan, L., Nilpanit, N., Arunyanart, P., Kim, P. V., Du, P. V. (2004) Applying rice seed-associated antagonistic bacteria to manage rice sheath blight in developing countries. *Plant Dis*. **88** (5): 557-564.

Mohamed, K. R. (2007) Environmental fate of imidazolinone herbicides and their Enantiomers in soil and water, Ph.D thesis University of Adelaide South Australia.

Mohd, A., Ramji, S., Sengar, R. S., Mehi, L., Gopal, S., Prashant, M., Sachan, S. K. (2014) Evaluation of different fungal and bacterial Bioagents against *Rhizoctonia solani* causing sheath blight of rice. *Biotech. Today*. **4** (2): 337.

Moore, S. J., Hill, N., Ruiz, C., Cameron, M. M. (2007) Field evaluation of traditionally used plant-based insect repellents and fumigants against the malaria vector *Anopheles darlingi* in Riberalta, Bolivian Amazon. *J. Med. Entomol*. **44** (4): 624-630.

Mordue, L. A. J., Morgan, E. D., Nisbet, A. J. (2005) Azadirachtin, a natural product in insect control. In '*Comperhensive molecular insect science'*, L. I., Giblbert, K., Iatrou., S. S., Gill (Eds), *Elsevier, oxford* P117-135.

Mossini, S. A. G., Arrotéia, C. C., Kemmelmeier, C. (2009) Effect of neem leaf extract and neem oil on *Penicillium* Growth, Sporulation, Morphology and ochratoxin A Production. *Toxins* **1** (1): 3-13.

Muller, R. H., Keck, C. M. (2004) Challenges and solutions for the delivery of biotech drugs–a review of drug nanocrystal technology and lipid nanoparticles. *J. Biotechnol.* **113** (1-3): 151-170.

Murphy, K. (2008) Nanotechnology: Agriculture's next "Industrial" revolution. *Spring, Willis* (Financial partner, Yankee farm credit, ACA), PP 3-5.

Nakahara, K., Alzorehy, N. S., Yoshihashi, Tadashi., Nguyen, H. T. T., Trakoontivakorn, G. (2003) Chemical composition and antifungal activity of essential oil from *Cymbopogon nardus* (Citronella grass). *JARQ*. **37** (4): 249-252.

Nakahara, K., Alzoreky, N. S., Yoshihashi, T., Nguyen, H. T. T., Trakoontivakorn, G. (2003) Chemicals composition and antifungal activity of essential oil from Gymbopogon nardus (Citronella grass). *Jpn. Agric. Res. Q*. **37** (40): 249-252.

Narayan, P. (2012) Neem-papaya juice passes dengue test. Available from: http://articles.timesofindia.indiatimes.com/12-20/chennai/35933070_1_dengue-virus-dengue-cases-denguepatients. [Accessed on 10 September, 2012].

Nathan, S. S., Kalaivani, K., Murugan, K. (2005) Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Acta Trop.* **96** (1): 47-55.

Nerio, L. S., Olivero-Verbel, J., Stashenko, E. (2010) Repellent activity of essential oils: A review. *Bioresour. Technol*. **101** (1): 372-378.

Nogueira, J. H. C, Gonçalez, E., Galleti, S. R., Facanali, R., Marques, M. O. M., Felício, J. D. (2010) Ageratum conyzoides essential oil as aflatoxin suppressor of *Aspergillus flavus*. *Int. J. Food Microbiol.* **137** (1): 55-60.

Oerk, E. C. (2006) Crop losses to pests. *J. Agric. Sci*. **144** (1): 31-43.

Okumu, F. O., Knols, B. G. J., Fillinger, U. (2007) Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector Anopheles gambiae. *Malar. J*. 6:63.

Oliveira, J. L. D., Campos, E. V. R., Bakshi, M., Abhilash, P. C. Fraceto, L. F. (2014) Application of nanotechnology for the encapsulation of botanical

insecticides for sustainable agriculture: Prospects and promises. *Biotech. Adv*. **32** (8): 1550-1561.

Oussalah, M., Caillet, S., Saucier, L., Lacroix, M. (2006) Antimicrobial effects of selected plant essential oils on the growth of a *Pseudomonas putida* strain isolated from meat. *Meat Sci.* **73** *(*2): 236-244.

Pankaj., Shakil, N. A., Kumar, J., Singh, M. K., Singh, K. (2012) Bioefficacy evaluation of controlled release formulations based on amphiphilic nano-polymer of carbofuran against *Meloidogyne incognita* infecting tomato. *J. Environ. Sci. Health, Part B*. **47** (6): 520-528.

Park, M., Lee, C. I., Seo, Y. J., Woo, S. R., Shin, D., Choi, J. (2010) Hybridization of the natural antibiotic, cinnamic acid with layered double hydroxides (LDH) as green pesticide. *Env. Sci. Pol. Res*. **17** (1): 203-209.

Parmar, B. S., Walia, S. (2001) Prospects and problems of phytochemicla biopesticides. In 'phytochemical biopesticides' Koul, O., Dhaliwal, G. S (Eds) Harwood Academic publishers, Amsterdam, P 133-210.

Paul, R., Prasad, M., Sah, N. K. (2011) Anticancer biology of *Azadirachta indica* (neem): a mini review. *Cancer Biol. Ther*. **12** (6): 467-476.

Paulsen, B. S. (2010) Highlights through the history of plant medicine. *Proceeding of the NASL*. pp. 18-29.

Pinheiro, P. F., Queiroz, V. T. D., Rondelli, V. M., Costa, A. V., Marcelino, T. D. P., Pratissoli, D. (2013) Insecticidal Activity of Citronella Grass essential oil on *Frankliniella schultzei* and *Myzus persicae*. *Ciênc. agrotec., Lavras.* **37** (2): 138-144.

Pu, Z., Zhang, Y. Y., Xu, Z. J., Jia, R., Lu, Y., Yang, F. (2010) Antibacterial activity of 9-octadecanoic acid-hexadecanoic acidtetrahydrofuran-3, 4-diyl ester from neem oil. *Agr. Sci. China*. **9** (8): 1236-1240.

Qiu, D. P., Li, Y., Fu, X., Jiang, Z., Zhao, X., Wang, T., Hou, W. (2009) Controlled-release of avermectin from organically modified hydrotalcite-like compound nanohybrids. *Chinese J. Chem*. **27** (3): 445-451.

Rana, V. S., Das, M., Blazqeuz, M. A. (2016) Essential oil yield, chemical composition, and total citral content of nine cultivars of Cymbopogon Species from Western India. *J. Herbs Spices, Med Plants.* ***22*** (4): 1540-3580.

Reddy, S. R., Fogler, H. S. (1981) Emulsion stability: determination from turbidity. *J. Colloid Interface Sci.* **79** (1): 101-104.

Reiss, H. (1975) Entropy-induced dispersion of bulk liquids. *J. Colloid Interface Sci.* **53** (1): 61–70.

Riyajan, S. A., Sakdapipanich, J. T. (2009) Development of a controlled release neem capsule with a sodium alginate matrix, cross linked by glutaraldehyde and coated with natural rubber. *Polym. Bull*. **63** (4): 609-22.

Rohini, B., Sandeep, K., Supriya, A. (2015) Application of Nanotechnology in Agriculture. *Trends in Biosci.* **8** (10): 2647-2649.

Roy, S., gurusubramanian, G. (2011) Bioefficacy of azadirachtin content formulation against three major sucking pests of tea in sub Himalayan tea plantation of north Bengal, India. *Agricultura Tropica Et Subtropica*, **44** (3): 134-143.

Sabri, M. A., Aslam, M. S., Hussain, D., Saleem, M. (2016) Evaluation of lethal response of biorational insecticides against *Spodoptera litura* (Lepidoptera: Noctuidae). *J. Entomol. Zool. Stud*. **4** (4): 270-274.

Saha, S., Galhardi, L. C. F., Yamamoto, K. A., Linhares, R. E. C., Bandyopadhyay, S. S., Sinha, S., Nozawa, C., Ray, B. (2010) Water-extracted polysaccharides from *Azadirachta indica* leaves: Structural features, chemical modification and anti-bovine herpesvirus 1 (BoHV-1) activity. *Int. J. Biol.Macromol*. **47** (5): 640-645.

Sahayarij, K., Selvarj, P., Balasubramanian, R. (2007) Cell mediated immune response of *Helicoverpa armigera* Hubner and *Spodoptera litura* Fabricius to farn phtoecdysteron. *J. Entomol*. **4** (4): 289-298.

Sakulku, U., Nuchuchua, O., Uawongyart, N., Puttipipatkhachorn, S., Soottitantawat, A., Ruktanonchai, U. (2009) Characterization and mosquito repellent activity of citronella oil nanoemulsion. *Int. J. Pharm*. **372** (1-2): 105-111.

Salma, U., Chen, N., Richter, D. L., Filson, P. B., Dawson-Andoh, B., Matuana, L., Heiden, P. (2010) Amphiphilic Coe/Shell nanoparticles to reduce biocide leaching from treated wood, 1-Leaching and biological efficacy. *Macromol. Mater. Eng.* **295** (5): 442-450.

Sarkar, D. J., Kumar, J., Shakil, N. A., Walia, S. (2012) Release kinetics of controlled release formulations of thiamethoxam employing nanoranged

amphiphilic PEG and diacid based block polymers in soil. J. *Environ. Sci. Health, Part A.* **47** (11*)*: 1701-1712.

Sarpeleh, A., Sharifi, K., Sonbolkar, A. (2009) Evidence of antifungal activity of wild rue (*Peganum harmala* L.) on phytopathogenic fungi. *J. Plant Dis Protect.* **116** (5): 208-213.

Satya, V. (2007) Neem genetic diversity in India and its use as biopesticide and biofertilizer. *Indian J. Pl. Protect.* **35** (2): 185-119.

Saxena, R. C. (1998) Botanical pest control. In: Dhaliwal, G. S., Heinrichs, E. A. (Eds.) Critical Reviews in Insect Pest Management. Common wealth Publishers, New Delhi, India, pp. 155-179.

Saxena, V., Hasan, A., Sharma, S., Pandey, L. M. (2017) Edible oil nanoemulsion: An organic nanoantibiotic as a potential biomolecule delivery vehicle. *Int. J. Polym. Mater*. 1-10.

Schluter, U., Bidmon, H. J., Grewe, S. (1985) Azadirachta affects growth and endocrine events in larvae of the tabacco hornworm, *Manduca sexta*. *J. Insect Physiol.* **31** (10): 773-777.

Schmutterer, H. (2002) In: The Neem Tree, second ed. Neem Foundation, Mumbai, India. **111**: 85.

Scott, N., Chen, H. (2002) Nanoscale science and engineering for agriculture and food systems. Report submitted to Cooperative State Research, *Education and Extension Service of the U.S. Department of Agriculture.* Retrieved fromhttp://www.nseafs.cornell.edu/web.roadmap.pdf.

Sehajpal, A., Arora, S., Kaur, P. (2009) Evaluation of plant extracts against *Rhizoctonia solani* causing sheath blight of rice. *J. Pharm. Pharm. Sci.* **1** (1): 25-30.

Shafiq, S., Shakeel, F., Talegaonkar, S., Ahmad, F. J., Khar, R. K., Ali, M. (2007) Development and bioavailability assessment of ramipril nanoemulsion formulation. *Eur. J. Pharm. Biopharm*. **66**: (2): 227-243.

Shah, M. A., Wani, S. H., Khan, A. A. (2016) Nanotechnology and insecticidal formulations. *Journal of Food Bioengineering and Nanoprocessing.* **1** (3): 285 -310.

Shakil, N. A., Pandey, A., Singh, M. K., Kumar, J., Awasthi, S. K., Pankaj., Srivastava, C., Singh, M. K., Pandey, R. P. (2010) Synthesis and

bioefficacy evaluation of new 3-substituted-3, 4-dihydro-1, 3-benzoxazines. *J. Environ. Sci. Health B.* **45** (2): 108-115.

Shakil, N. A., Singh, M. K., Pandey, A., Kumar, J., Pankaj, Parmar, V. S., Singh, M. K., Pandey, R. P., Watterson, A. C. (2010) Development of poly (ethylene glycol) based amphiphilic copolymers for controlled release delivery of carbofuran. *J. Macromol. Sci. Pure Appl. Chem. Part A.* **47** (3): 241-247.

Sharma, N., Tripathi, A. (2008) Effects of *Citrus sinensis* (L.) *Osbeck epicarp* essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiol. Res*. **163** (1): 337-344.

Sharma, V., Walia, S., Dhingra, S., Kumar, J., Parmar, B. S. (2006) Azadirachtin-A and tetrahydroazadirachtin-A concentrates: preparation, LC-MS characterization and insect antifeedant/IGR activity against *Helicoverpa armigera* (Hubner). *Pest Manag Sci*. **62** (10): 965-975.

Shivpuri, A., Gupta, R. B. L. (2001) Evaluation of different fungicides and plant extracts against *Sclerotinia sclerotiorum* causing stem rot of mustard. *Phytopathology*. **52**: 272-274.

Singh, K., Tarannum, A., Sharma, D., Chandola, P. (2015) Antifungal Activity of *Azadirachta indica* leaf extract against Phytopathogenic Fungi (*R. solani*). *Trends in Biosciences* **8** (17): 4483-4486.

Singhal, S., Bhatt, M. (2016) Review on a natural drugstore- neem. *Int. J. Pharm. Pharm. sci.* **5** (8): 379-396.

Sneh, B., Burpee, L., Ogoshi, A. (1991) Identification of Rhizoctonia species, APS Press, St. Paul, Minnesota, USA.

Solans, C., Solé, I. (2012) Nanoemulsions: Formation by low-energy methods. *Curr. Opin. Coll. Interf. Sci.* **17** (5): 246-254.

Song, S. L., Liu, X. H., Jiang, J., Qian, Y., Zhang, N., Wu, Q. H. (2009) Stability of triazophos in self-nanoemulsifying pesticide delivery system. *Coll. Surf. A: Physicochem. Eng. Aspects.* **350** (1-3**)**: 57-62.

Subapriya, R., Nagini, S. (2005) Medicinal properties of neem leaves: a review. *Curr. Med. Chem. Anticancer Agents,* **5** (2): 149-160.

Sudhakaran, R. (2002) Efficacy of lufenuron (Match 5% EC) against *Spodoptera litura* under *in vitro* condition**.** *Insect Environ.* **8:** 47-48.

Suharti, W. S., Nose, A., Zheng, S. H. (2016) Metabolomic study of two rice lines infected by *Rhizoctonia solani* in negative ion mode by CE/TOF-MS. *J. Plant Physiol*. **1** (206): 13-24.

Suresh, U., Murugan, K., Panneerselvam, C., Rajaganesh, R., Roni, M., Aziz, A., Al- Aohd, H. A. N., Trivedi, S., Rehman, H., Kumar, S., Higuchi, A., Canaleg, A., Benelli, G. (2017) *Suaeda maritima*-based herbal coils and green nanoparticles as potential biopesticides against the dengue vector *Aedes aegypti* and the tobacco cutworm *Spodoptera litura*. *Physiol. Mol. Plant Pathol.* 1-11.

Szczepanik, M., Obara, R., Szumny, A., Gabrys, B., Halarewicz-Pacan, A., Nawrot, J., Waarzenczvk, C. (2005) Synthesis and Insect Antifeedant Activity of Precocene Derivatives with Lactone Moiety. *J. Agric. Food Chem*. **53** (15): 5905–5910

Tabassum, N., Vidyasagar, G. M. (2013) Antifungal investigations on plant essential oils. A review. *Inter. J. Pharm. Sci*. **5** (2): 19-28.

Tak, J. H., Isman, M. B. (2016) Metabolism of citral, the major constituent of lemongrass oil, in the cabbage looper, *Trichoplusia ni*, and effects of enzyme inhibitors on toxicity and metabolism. *Pestic. Biochem. Physiol*. 133: 20-25.

Thas, J. J. (2008) Medicine background and principles and the application for skin diseases. *Clin. Dermatol*. **26** (1): 62-78.

Tiwari, R., Verma, A. K., Chakraborty, S., Dhama, K., Singh, S. V. (2014) Neem (*Azadirachta indica*) and it is potential for safe guarding health of animals and humans: A reviews. *J. Bio. Sci.* **14** (2): 110-123.

Tiwari, R., Verma, A. K., Chakraborty, S., Singh, S. V. (2014) Neem and it is Potential for Safeguarding Health of Animals and Humans: *A Review. J. Biol. Sci. Faisalabad*. **14** (2) : 110-123.

Tiwari, V., Darmani, N. A., Yue, B. Y. J. T., Shukla, D. (2010) *In vitro* antiviral activity of neem (*Azardirachta indica* L.) barks extract against herpes simplex virus type-1 infection. *Phytother Res*. **24** (8): 1132-1140.

Trigo, C., Celis, R., Hermosin, M. C., Cornejo, J. (2009) Organo clay-based formulations to reduce the environmental impact of the herbicide diuron in olive groves. *Soil Sci. Soc. Am. J*. **73** (5): 1652-1657.

Trigo, C., Koskinen, W. C., Celis, R., Sadowsky, M. J., Hermosin, M. C., Cornejo, J. (2010) Bioavailability of organoclay formulations of atrazine in soil. *J. Agric. Food Chem.* **58** (22): 11857-11863.

Trongtokit, Y., Rongsriyam, Y., Komalamisra, N., Apiwathnasorn, C. (2005) Comparative repellency of 38 essential oils against mosquito bites. *Phytother. Res.* **19** (4): 303-309. .

Vattikonda, R. S., Sangam, S. R (2016) Assessment of antifeedant potential of azadirachtin, a plant product of *Azadirachta indica* against *Papilio demoleus* L. (Lepidoptera: Papilionidae) larvae. *J. Entomol. Zool. Stud.* **4** (5): 956-959.

Veeraraghavan, J., Natarajan, M., Lagisetty, P., Awasthi, V., Herman, T. S., Aravindan, N. (2011) Impact of curcumin, raspberry extract, and neem leaf extract on rel protein-regulated cell death/radiosensitization in pancreatic cancer cells. *Pancreas,* **40** (7): 1107-1119.

Vincent, J. M. (1947) Distortion of fungal hyphae in the presence of certain inhibitors. *Nature,* **159** (4051): 850.

Wang, J., Li, J., Cao J., Jiang, W. (2010). Antifungal activities of neem (*Azadirachta indica*) seed kernel extracts on postharvest diseases in fruits. *Afr. J. Microbiol. Res.* **4** (11): 1100-1104.

Wang, L., Lia, X., Zhanga, G., Donga, J., Easoeb, J. (2007) Oil-in-water nanoemulsions for pesticide formulations. *J. Colloid. Int. Sci*. **314** (1): 230-235.

Wany, A., Kumar, A., Nallapeta, S., Jha, S., Nigam, V. K., Pandey, D. M. (2014) Extraction and characterization of essential oil components based on geraniol and citronellol from Java citronella (*Cymbopogon winterianus* Jowitt). *Plant Growth Regul*. **73** (2): 133-145.

Woodhall, J. W., Lees, A. K., Edwards, S. G., Jenkinson, P. (2007) Characterization of *Rhizoctonia solani* from potato in Great Britain. *Plant Pathol*. **56** (2): 286-295.

Wu, Q. J., Zhao, J. Z., Taylor, A. G., Shelton, A. M. (2006) Evaluation of Insecticides and Application Methods against *Contarinia nasturtii* (Diptera: Cecidomyiidae). A new invasive Insect Pest in the United States. *Econ. Entomol.* **99** (1): 117-122.

Xu, J., Song, X., Yin, ZQ., Cheng, A. C., Jia, R.Y., Deng, Y. X., Ye, K. C., Shi, C. F., Lv, C., Zhang, W. (2012) Antiviral activity and mode of action of extracts from need seed kernel against duck plague virus *in vitro*. *Poult. Sci*, **91** (11): 2802-2807.

Yang, F., Li, X., Zhu, F., Lei, C. (2009) Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *J. Agric. Food Chem.* **57** (21): 10156-10162.

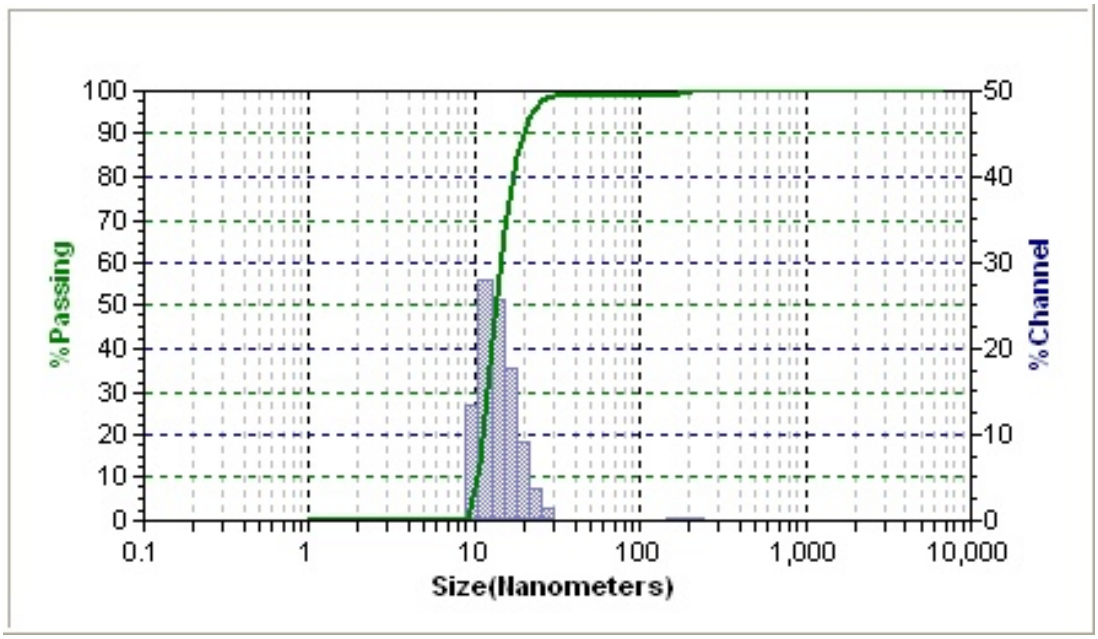
Zeng, H., Li, X. F., Zhang, G., Dong, J. F. (2008) Preparation and characterization of beta cypermethrin nanosuspensions by diluting O/W microemulsions. *J. Disp. Sci. Tech.* **29** (3): 358-361.

Zhang, Y. Q., Xu, J., Yin, Z. Q., Jia, R.Y., Lu, Y., Yang, F., Du, Y. H., Zou, P., Lv, C., Hu, T. X., Liu, S. L., Shu, G., Yi, G. (2010) Isolation and identification of the antibacterial active compound from petroleum ether extract of neem oil. *Fitoterapia*, **81** (7): 747-750.

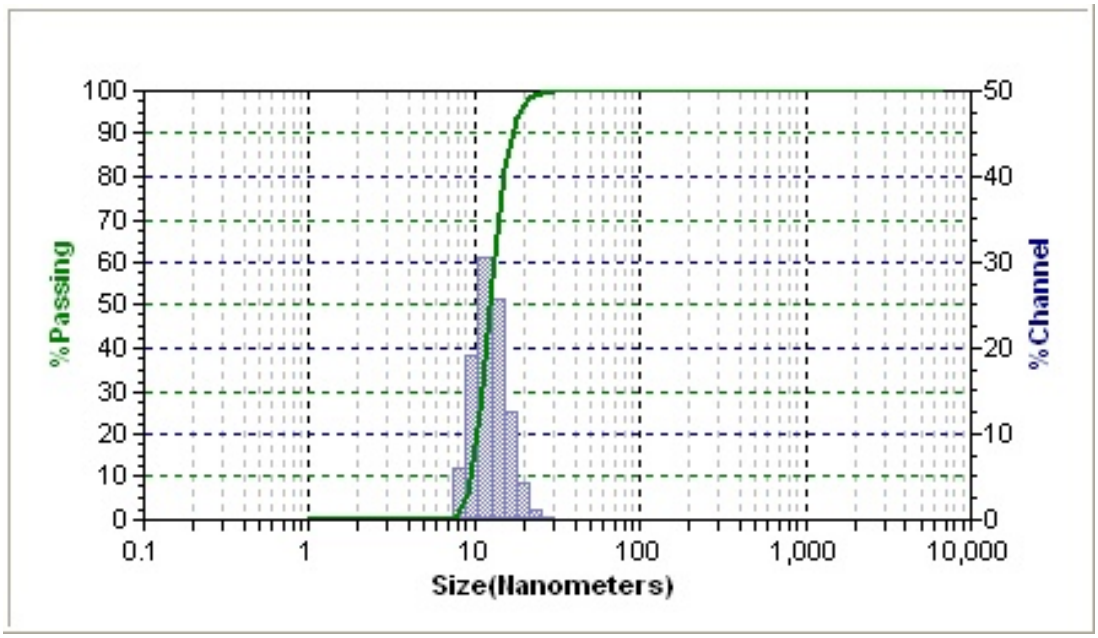
Zheng, A., Lin, R., Zhang, D., Qin, P., Xu, L., Ai, P., Ding, L., Wang, Y., Chen ,Y., Liu, Y., Sun , Z., Feng, H., Liang, X., Fu, R., Tang, C., Li, Q., Zhang, J., Xie, Z., Deng, Q., Li, S., Wang, S., Zhu, J., Wang, L., Liu, H., Li, P. (2013) The evolution and pathogenic mechanisms of the rice sheath blight pathogen. *Nat. Commun*. **4**: 1424.

Ziani, K., Chang, Y., McLandsborough, L., McClements, D. J. (2011) Influence of surfactant charge on antimicrobial efficacy of surfactant stabilized thyme oil nanoemulsions. *J. Agric. Food Chem*. **59** (11): 6247-6255.

Zibaee, I., Farahani, H. M., Razi, J. M., Moghaddam, M. N. (2016) Synergistic effect of two medicinal plants in cream formulation on repellency of vector mosquito and rove beetle. *J. Entomol. Zoolo. Stud.* **4** (4): 73-79.



Micelle size of NNE-1 (PEN+0.5% Citronella oil) as measured by DLS



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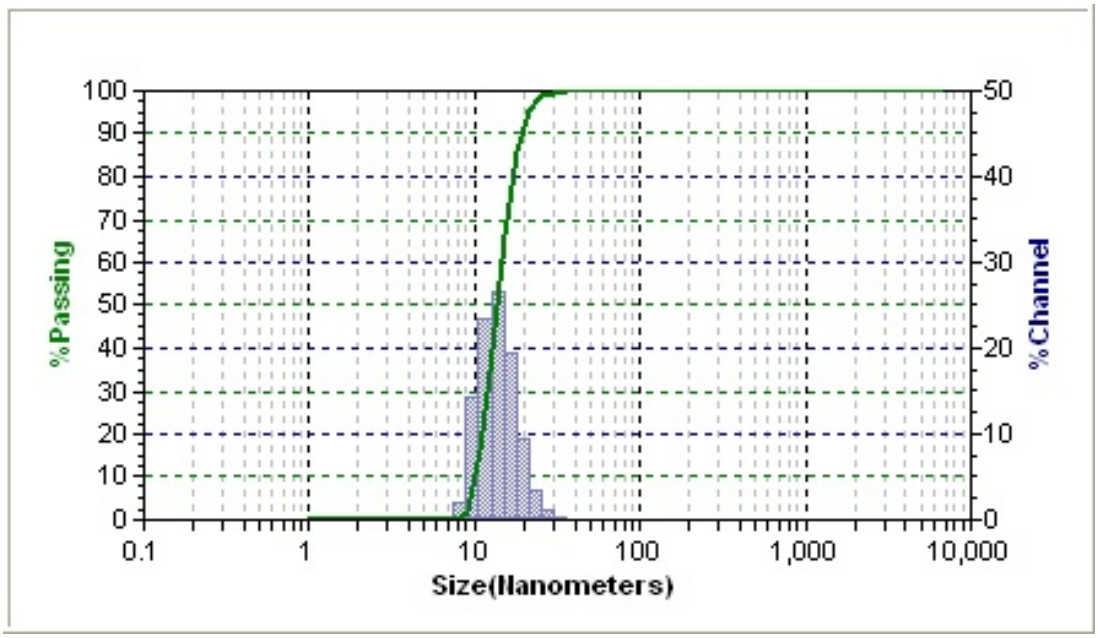

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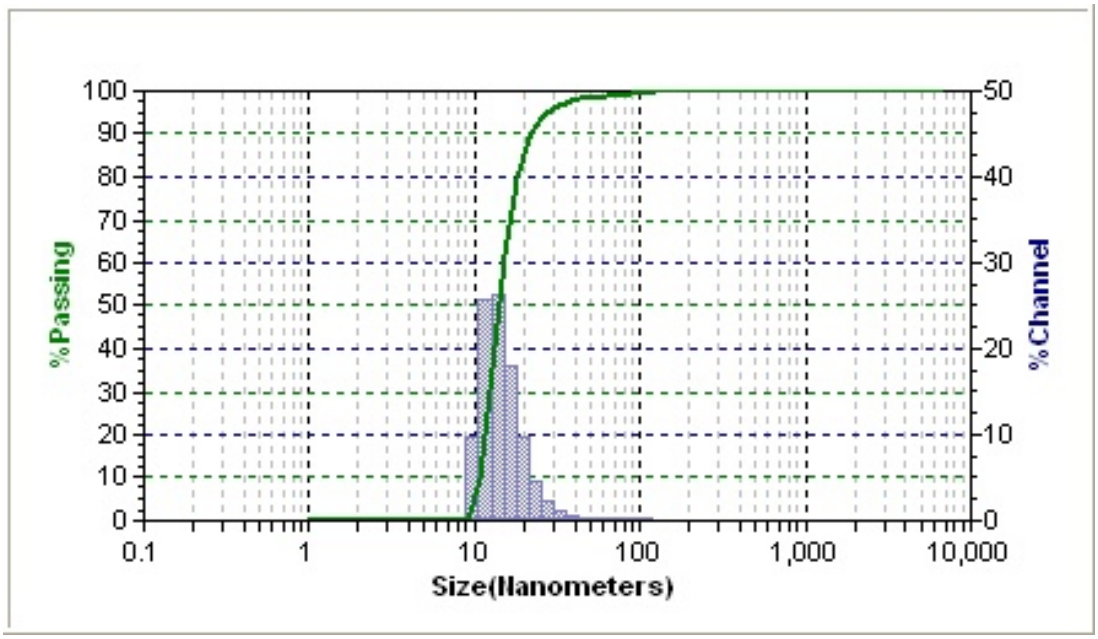

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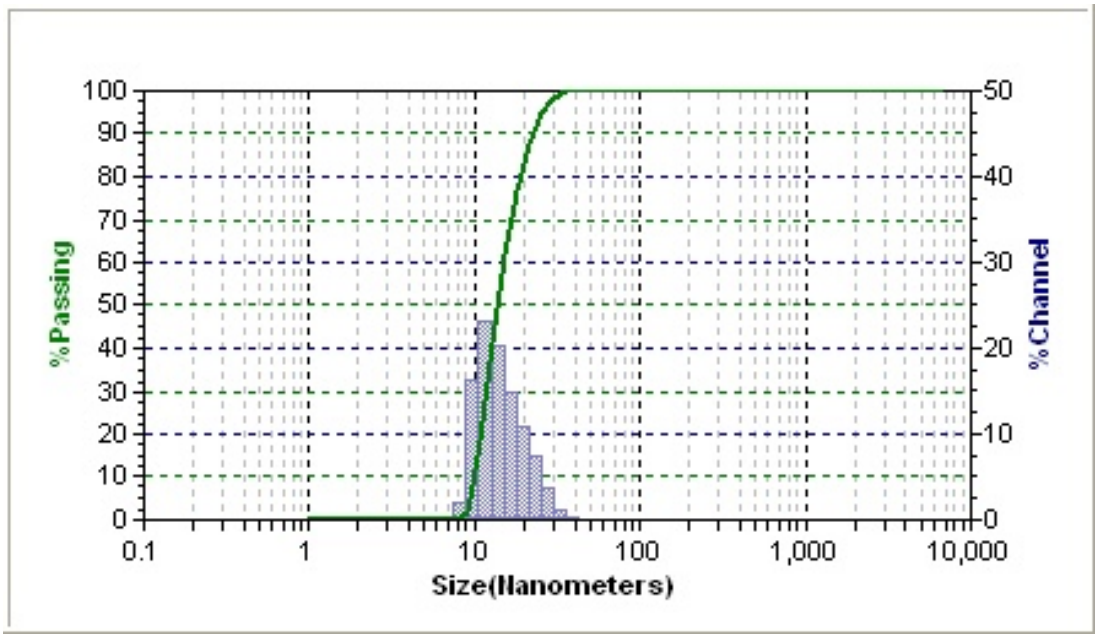

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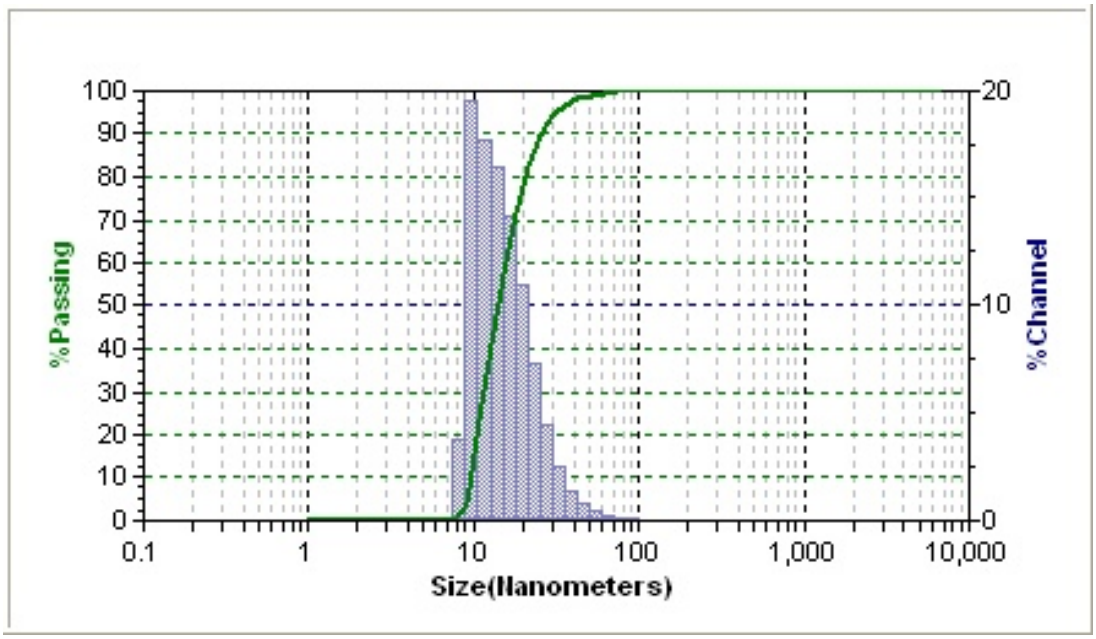

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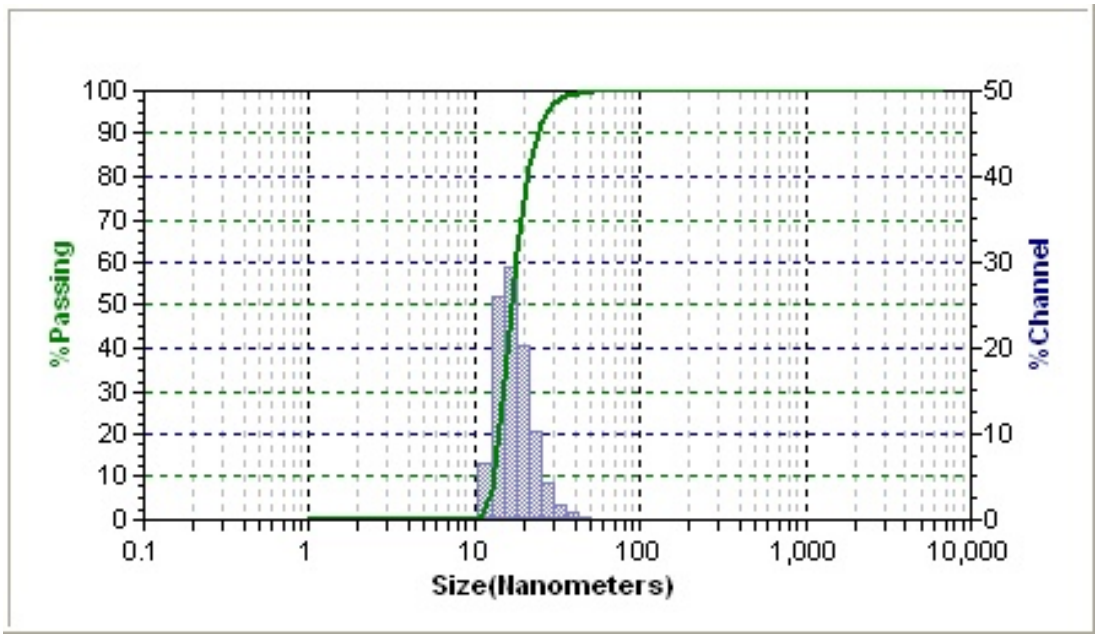

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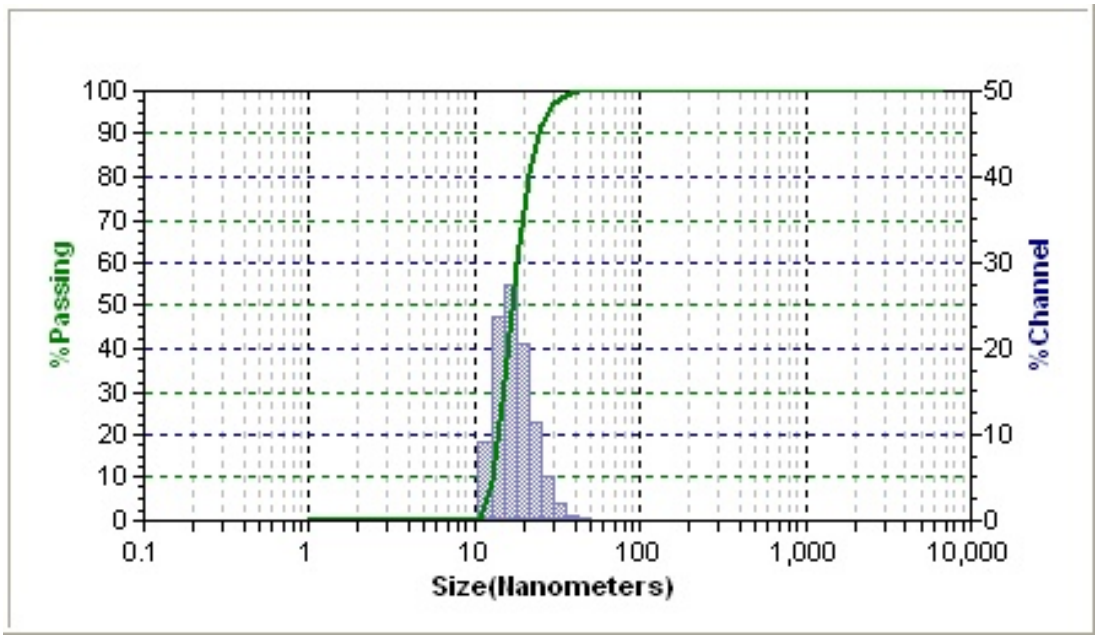

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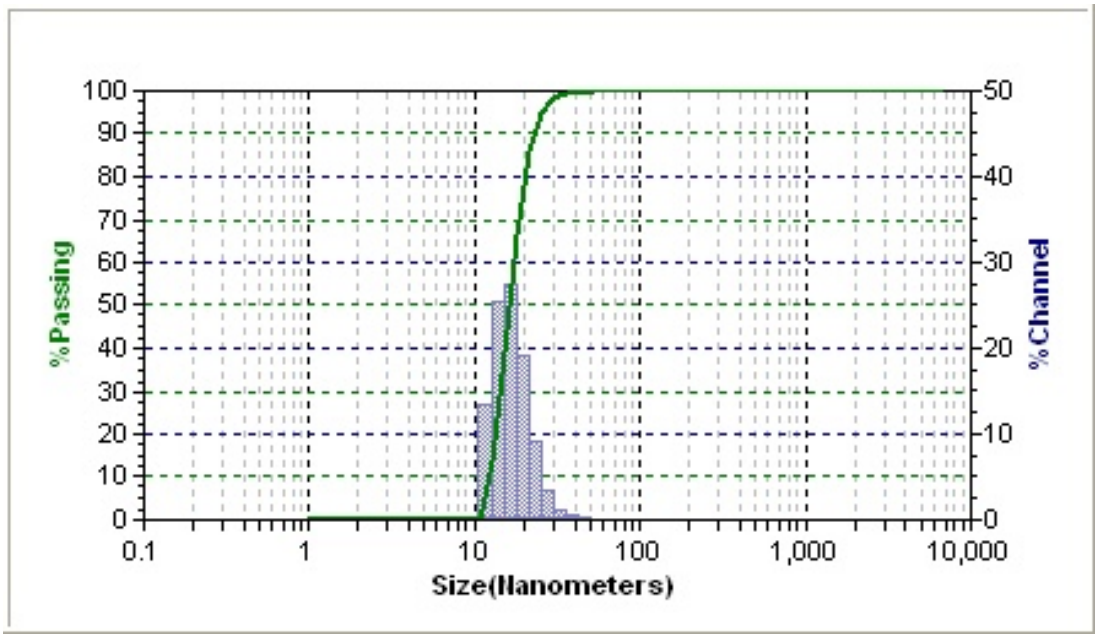

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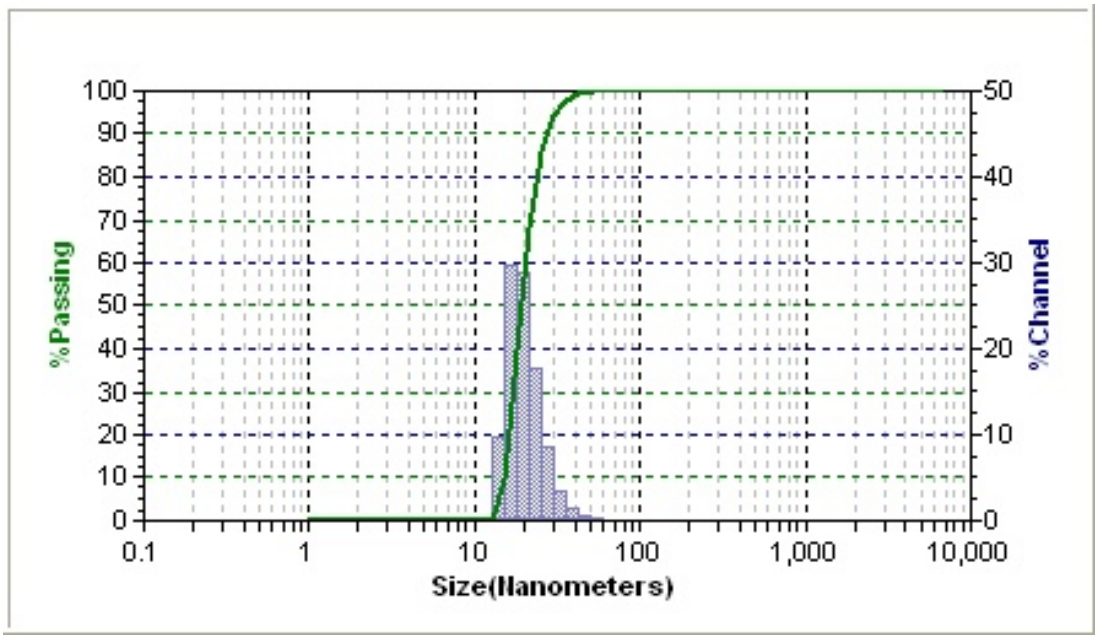

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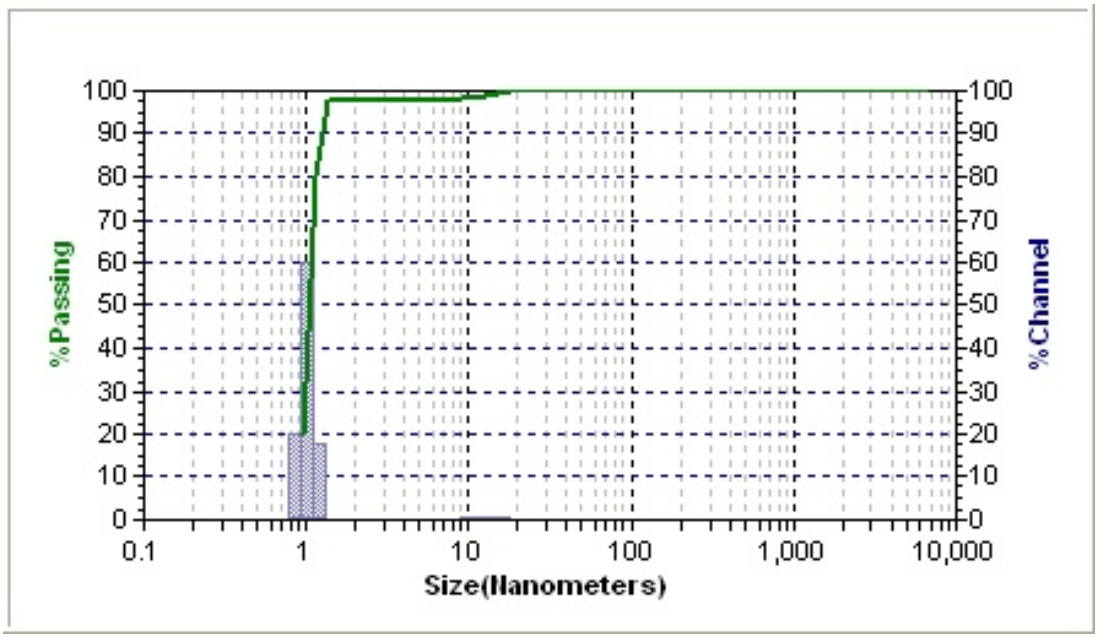

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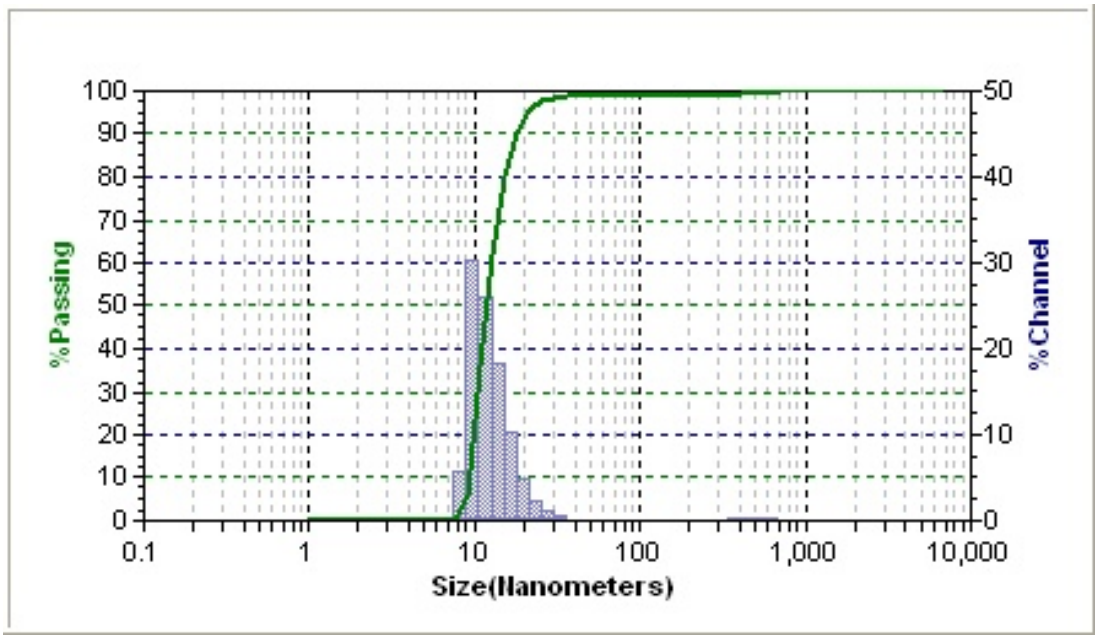

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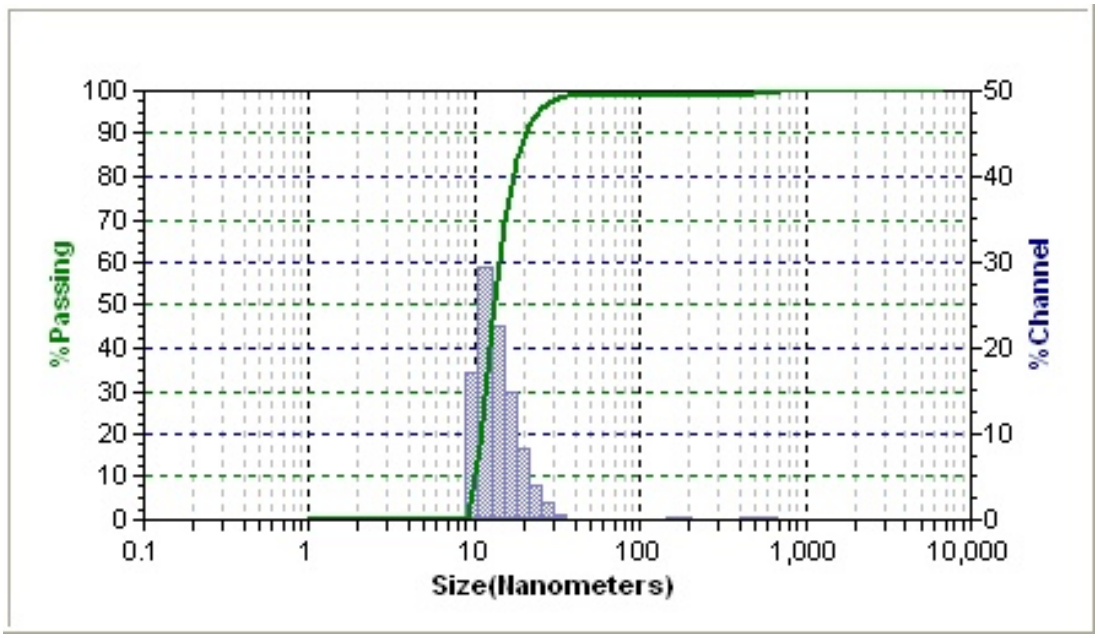

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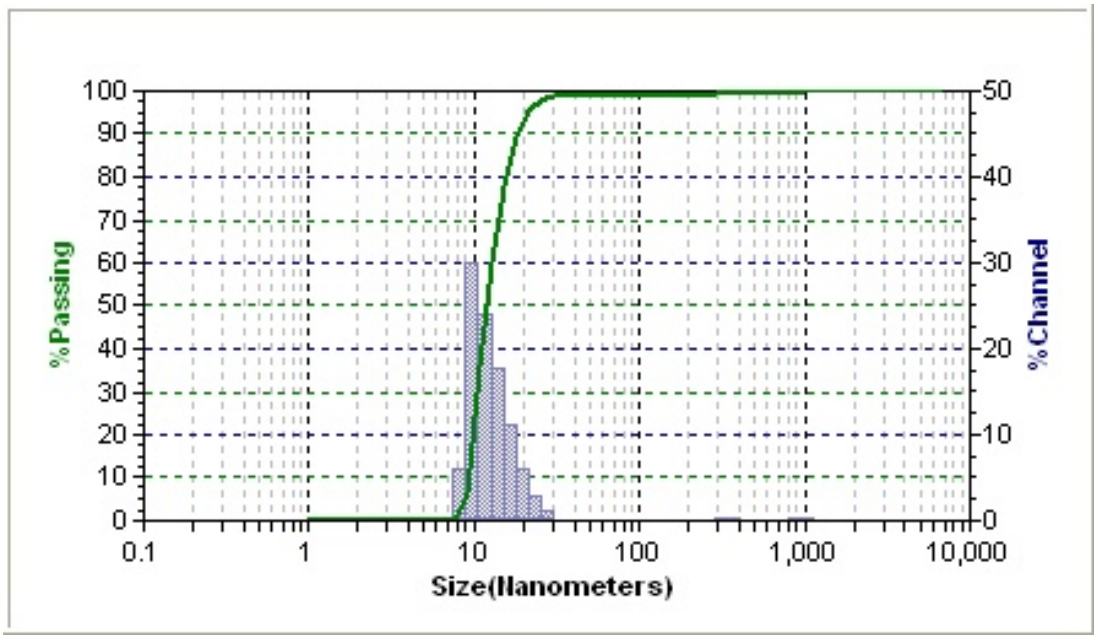

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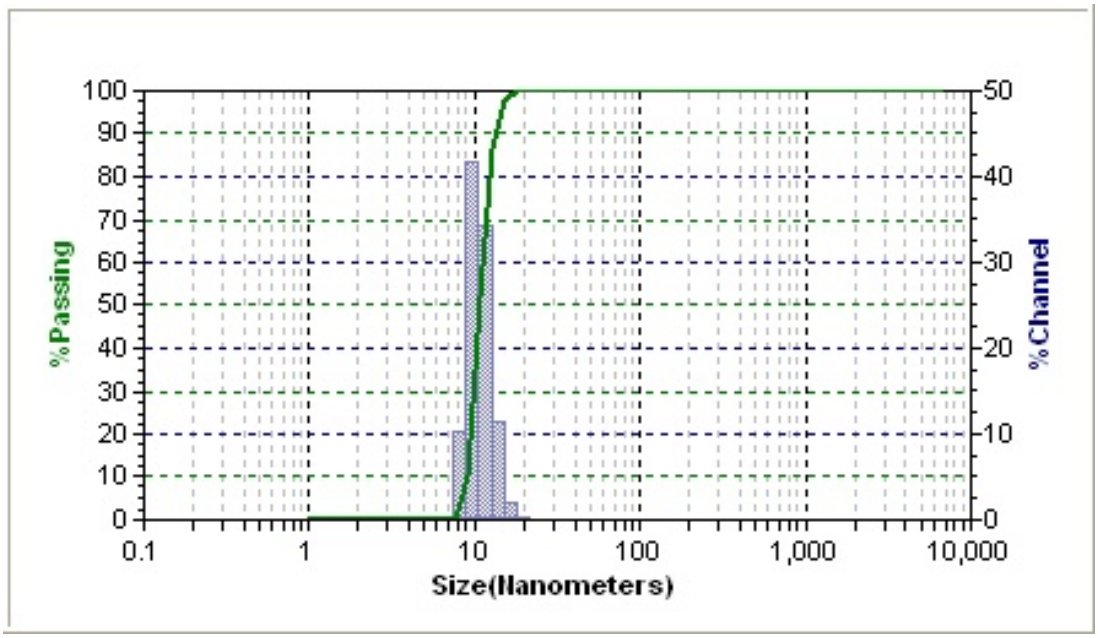

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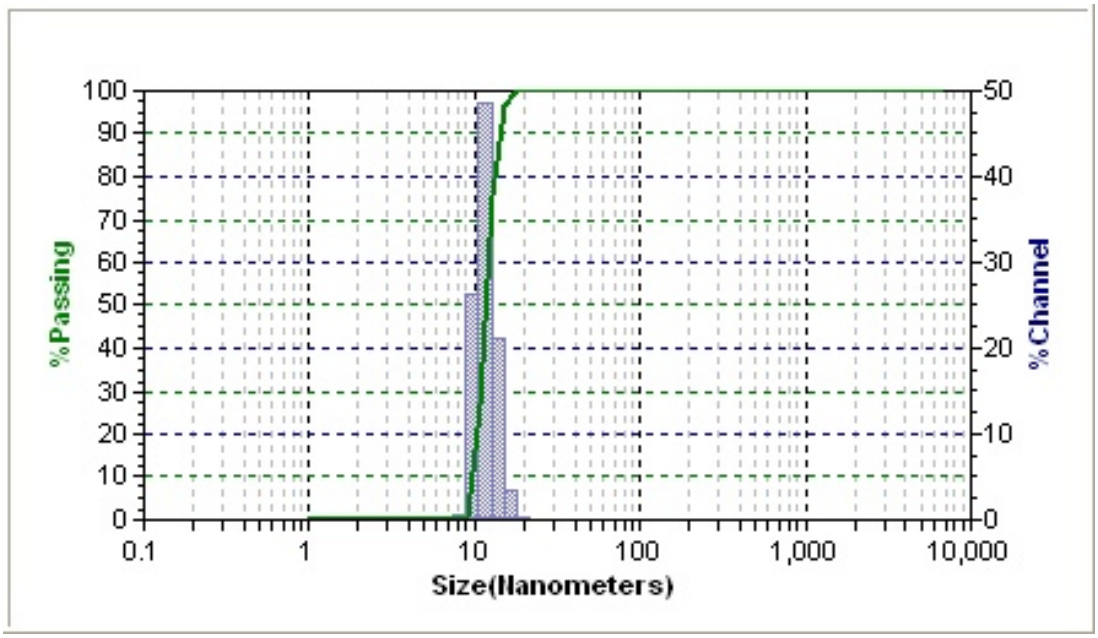
Micelle size of CNE-6 (PEN+3.0% Neem oil) as measured by DLS



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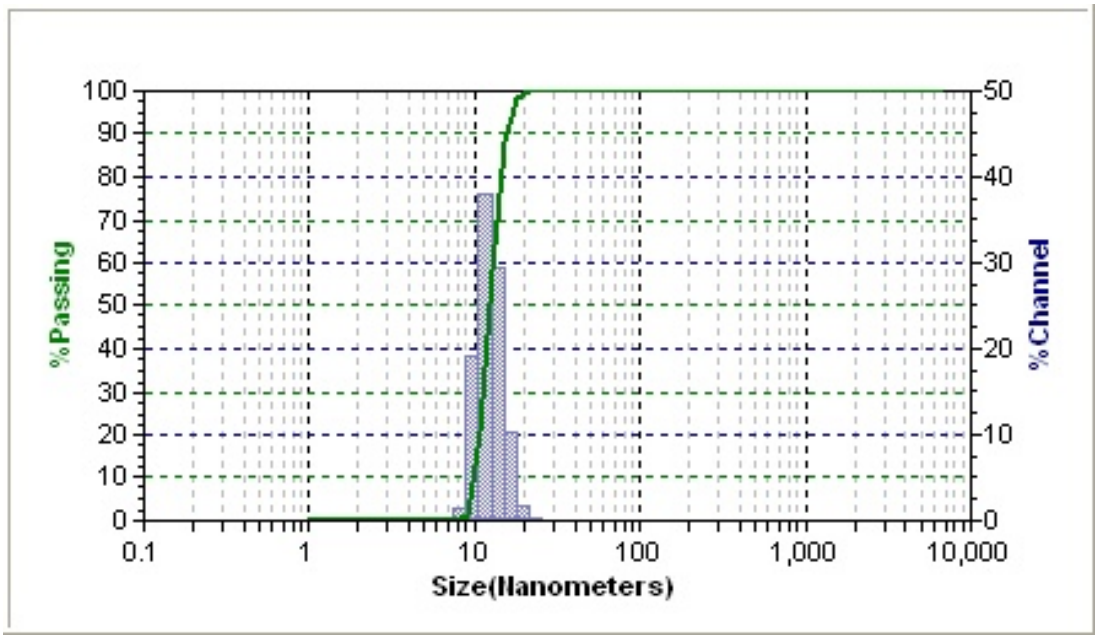

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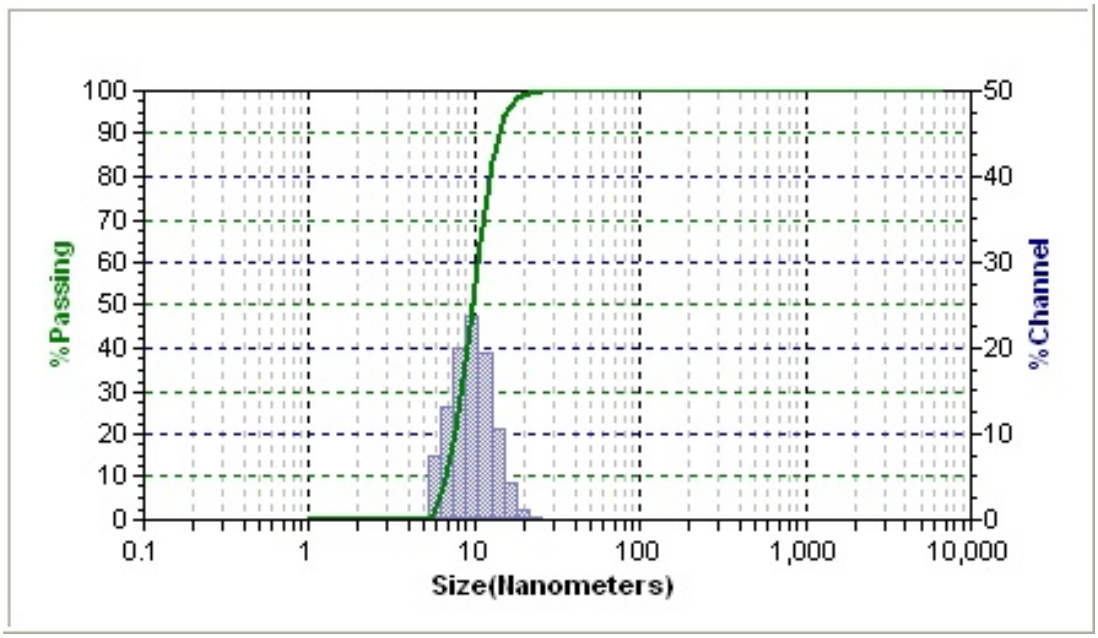

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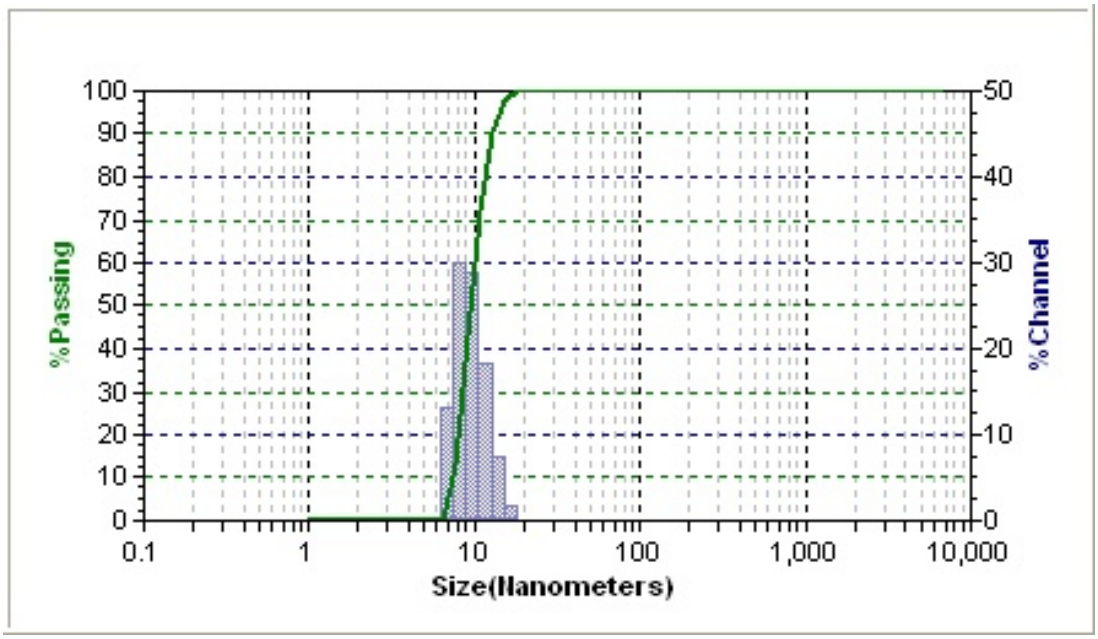

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