

**MOSQUITOCIDAL EFFICACY & TOXICITY
PROFILING OF PHYTOCHEMICALS
LOADED NANOPARTICLES**

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by

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Submitted

**in fulfilment of the requirements of the degree of Doctor of Philosophy
to the**



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Dedicated
To
The Almighty
&
My Beloved family

CERTIFICATE

This is to certify that the thesis entitled “**Mosquitocidal Efficacy & Toxicity Profiling of Phytochemicals Loaded Nanoparticles**” submitted by **Ms. Smriti Kala** to Indian Institute of Technology Delhi for award of degree of **Doctor of Philosophy** has been prepared under our guidance with the rules and regulations of Indian Institute of Technology Delhi India. The research report results presented in this thesis have not been submitted for any degree or diploma in any other institute or university.



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ABSTRACT

The enormous global burden and impact of mosquito-borne diseases have challenged the health sector worldwide. Due to the unavailability of drugs and vaccines, mosquito controls remain an alternative solution. With increasing concern and harmful effects of synthetic chemical pesticides, there has been renewed interest in technologies based on bio-inspired products. In particular, plant-derived products rich in phytochemicals are effective and popularized as bio-pesticides due to their safety towards users and the environment. However, the ease with which plant-inspired pesticides degrades under realistic field conditions has hampered their broader applications.

Nanotechnology provides the basis for improving the effectiveness of bio-pesticides. The most promising nanostructured systems are *polymeric encapsulation*, *nanogel*, and *nanoemulsions*. These nanostructure systems can contribute towards sustainable vector management programs due to their potential for assisting in reduced doses of pesticides while at the same time improving stability and offering controlled release, depending upon the type of nanostructure. The bio-derived polymers chitosan and pectin are safe, biodegradable, non-toxic, and used as wall-forming encapsulating agents. The nanocapsules were formed using chitosan and pectin using the ionic gelation method, which involves the interactions of anionic polymer with oppositely charged ions to create hydrogel beads, also known as gel spheres, which are spherical cross-linked hydrophilic polymeric entities capable of swelling in water and the release of active is controlled by polymer relaxation. Pectin and chitosan produce electrostatically stabilized gel networks with divalent metal cations (calcium) and tripolyphosphate (TPP), respectively.

Additionally, these nanostructures raise concern regarding their non-target exposure since NP may endanger human health through oral, inhalation, and cutaneous exposure, which may interact with cells and tissues and alteration, can occur. The safety evaluation of nanostructures is vital to determine their potentially harmful effects. Considering all these facts, the present work was designed to develop phytochemicals loaded nanostructures, alias nanoparticles for controlling mosquito vectors and their toxicity assessments on *in vitro* mice model or *in the Vivo* cell line.

The screening of fixed oils viz. *Schleicher oleosa* (Kusum) oil, *Calophyllum inophyllum* (Tamanu) oil, Morigina oil; essential oils lemongrass oil, cedarwood oil; and bio-waste cashew nut shell liquid (CNSL) were evaluated for their efficacy against immature (larva) and adult mosquitoes before being developing them in nano-form. The Phyto-constituents of selected phyto-sourced were analyzed by GC-MS. After the successful screening and detailed Phyto-constituents analysis, the ones with the best performance were loaded into nanoparticles viz. nanocapsules, nanocapsules to nanogel, and nanoemulsion. The characterization of developed nanoformulations was done by TEM / DLS, and DSC was also performed where ever required. The formulations were observed for storage stability assessed by changes in physical appearance or particle size upon storage.

Further, the nanocapsules impregnated on a mini cotton bag to produce ready-to-use (RTU) formulation and nano-micellar emulsion were used for targeting immature mosquito stages. In contrast, nanogel was impregnated on cotton fabric to produce long-lasting and wash durable finished mosquito repellent fabric. The nanocapsules cannot react or bind with the fibers, so it is necessary to bind them to the fiber. Acrylates can be used to bind nanocapsules to fabric, which also prevents their washing off during a series of washing.

Chitosan nanocapsules, containing lemongrass (*Cymbopogon citratus*) oil (LGO) have been developed in gel form in which acrylate (Ac) was incorporated as a thickener and fabric binder. The gel was impregnated on fabric to achieve long-lasting and wash-durable mosquito repellency. FTIR and XRD were used to examine the interaction of cotton fibres with gel. Wash durability of gel was compared with chitosan nanocapsules without acrylate (LGO-encap) using SEM and GC-MS. The SEM analyses revealed that acrylate containing nanocapsules was retained on fabric after a series of washing. The GC-MS results indicated that the relative amount of deducible oil components from fabric was higher after the washing series in acrylate-containing nanocapsules (LGO-encap-Ac), which further points to the improved wash durability and retention of capsules on fabric. The bio-efficacy results of post-fifteen washing turned out was 75% of repellency against mosquitoes with the use of acrylate, while in nanocapsules without acrylate, only 51% of repellency was achieved. Furthermore, the 36 days repeated application of nanogel on Swiss albino mice did not show any signs of dermal toxicity. The formulation is, thus, suitable to impregnate the dress of the military personals and individuals who have to perform field duty and where the risk of mosquito bites is probably more.

The cedarwood (*Cedrus deodara*) essential oil embedded pectin nanocapsules were produced. The nanocapsules were characterized according to their morphology, size, encapsulation efficiency, and thermal stability. Furthermore, the nanocapsules were impregnated onto mini cotton tea bags to be employed as RTU (ready to use) formulation for treating the breeding sites of mosquitoes. The larvicidal activity of the bags treated with pectin-cedar wood nanocapsules was assessed against malaria vector, *Anopheles culicifacies*, and 98% mortality was recorded till four weeks; this

suggests its potential hassle-free applications in controlling mosquito vector. The Nanocapsules were found to be safe on the mouse cell line.

The cashew nut (*Anacardium occidentale*) shell liquid (CNSL) is produced as waste by the cashew nut processing industry. The disposal of this substantial waste product may become an issue in terms of ecological issues. CNSL has insecticidal properties; despite this, it has not been utilized to its full extent. CNSL is insoluble in water; in this context, we used CNSL to produce a nanoemulsion for larvicidal applications by employing the spontaneous emulsification technique. CNSL monodispersed micelles with a mean diameter of 52nm were produced. The comparative bioefficacy of bulk and nano CNSL against the third instar larva of *Anopheles culicifacies* was determined, with LC₅₀ values of 18.1mg/L and 1.4 mg/L, respectively. The scanning electron microscope was used to assess the larva's morphological damage after exposure to CNSL nanoemulsion. Histopathological examination revealed a detailed mode of toxicity of nano CNSL on the larva. The result obtained showed that the CNSL nanoemulsion has enhanced larvicidal activity compared to bulk CNSL, allowing effective waste utilization. The utilization of CNSL nanoemulsion may also resolve disposal-related issues of massive waste generated by the cashew nut industry. Such an approach may be suitable for enhanced efficacy against mosquito vectors at lower doses and contribute to reduced use of synthetic pesticides. The Nano micelle possesses dose-dependent cytotoxicity on the mice cell line.

सार

मच्छर जनित बीमारियों के भारी वैश्विक बोझ और प्रभाव ने दुनिया भर में स्वास्थ्य के क्षेत्र के समक्ष एक बड़ी चुनौती खड़ी कर दी है। दवाओं और टीकों की अनुपलब्धता के कारण मच्छर नियंत्रण के समाधान की तलाश जारी है। साथ ही सिंथेटिक (Synthetic) रासायनिक कीटनाशकों के प्रयोग के कारण हानिकारक प्रभावों से बढ़ती चिंता के फलस्वरूप, जैव-प्रेरित उत्पादों पर आधारित प्रौद्योगिकियों में रुचि भी बढ़ी है। विशेष रूप से फाइटोकेमिकल्स से भरपूर पादप व्युत्पन्न उत्पाद मच्छर नियंत्रण के लिए अधिक प्रभावी होते हैं। ये उत्पाद उपयोगकर्ता और पर्यावरण के प्रति सुरक्षित होने के कारण जैव-कीटनाशकों के रूप में लोकप्रिय होते जा रहे हैं। हालांकि, पौधे जनित कीटनाशकों का वास्तविक क्षेत्र की परिस्थितियों में आसानी से विघटन होने से इनके व्यापक अनुप्रयोगों में बाधा उत्पन्न होती है।

नैनो-प्रौद्योगिकी ने जैव-कीटनाशकों की प्रभावशीलता में उन्नति के लिए आधार प्रदान किया है। सर्वाधिक प्रभावी नैनोसंरचित प्रणालियाँ (nanostructured systems) *पॉलीमेरिक एनकैप्सुलेशन*, *नैनोजेल* और *नैनोइमल्शन* पर आधारित हैं। ये नैनोस्ट्रक्चर सिस्टम स्थायी वेक्टर प्रबंधन कार्यक्रम में महत्वपूर्ण योगदान दे सकते हैं। ये प्रणालियाँ कीटनाशकों की खुराक कम करने में सहायता करती हैं, साथ ही स्थिरता में सुधार और रिसाव को नियंत्रित करती हैं। जैव-व्युत्पन्न पॉलिमर जैसे काइटोसैन और पेक्टिन सुरक्षित, बायोडिग्रेडेबल और गैर-विषाक्त हैं तथा परत बनाने वाले इनकैप्सुलेटिंग एजेंटों के रूप में उपयोग किए जाते हैं। नैनोकैप्सूल का उत्पादन आयनिक जेल विधि के माध्यम से काइटोसैन और पेक्टिन का उपयोग करके किया गया था। आयनिक जेलेशन में विपरीत रूप से चार्ज किए गए आयन एक आयनिक बहुलक के साथ क्रिया करके हाइड्रोजेल बीड्स (Hydrogel Beads) का उत्पादन करते हैं जिन्हें जेलिस्फेयर भी कहा जाता है। ये जेलिस्फेयर, गोलाकार क्रॉस-लिंकड हाइड्रोफिलिक पॉलीमेरिक इकाई हैं जो पानी में फूलने में सक्षम हैं और सक्रिय तत्वों के रिसाव को नियंत्रित करती हैं। पेक्टिन और काइटोसैन, इलेक्ट्रोस्टैटिक रूप से स्थिर जेल नेटवर्क बनाते हैं, जैसे- डाइवैलेंट मेटल केशन (कैल्शियम) और ट्रिपोलीफॉस्फेट (टीपीपी) के साथ जेलिस्फेयर जेल का निर्माण।

इसके अतिरिक्त, नैनोस्ट्रक्चर के उत्पादन में उनके गैर-लक्षित जोखिम का आकलन करना एक मुख्य चरण है। क्योंकि नैना कण मानव स्वास्थ्य (मुख्वांग, साँस और त्वचीय रोग) और पर्यावरण के लिए हानिकारक हो सकते हैं। इस हेतु नैनोस्ट्रक्चर के संभावित खतरनाक दुष्प्रभाव तथा उनका सुरक्षा मूल्यांकन महत्वपूर्ण है। इन सभी तथ्यों को ध्यान में रखते हुए, वर्तमान कार्य को फाइटोकेमिकल्स लोडेड नैनोस्ट्रक्चर विकसित करने के लिए डिजाइन किया गया था। मच्छर वैक्टर एवं उनकी विषाक्तता को नियंत्रित करने वाले नैनोकणों का मूल्यांकन इन विट्रो चूहा मॉडल या इन विवो सेल लाइन में किया गया।

अपरिपक्व (लार्वा) और वयस्क मच्छरों के नियंत्रण के लिए नैनो उत्पाद विकसित करने से पहले, निश्चित तेलों जैसे *श्लीचेरा ओलियोसा* (कुसुम) तेल, *कैलोफिलम इनोफिलम* (तमानु) तेल, *मोरिंगिना* तेल, आवश्यक तेल जैसे *लेमनग्रास* तेल, देवदार का तेल और जैव-अपशिष्ट जैसे काजू खोल तरल (सीएनएसएल) का मूल्यांकन और उनकी प्रभावकारिता का अध्ययन किया गया। चयनित फाइटो-स्रोत के फाइटो-घटकों का विश्लेषण जीसी-एमएस द्वारा किया गया था। सफल स्क्रीनिंग और विस्तृत फाइटो-घटक विश्लेषण के बाद, सर्वश्रेष्ठ प्रदर्शन वाले अवयवों को नैनोकणों में लोड किया गया, जैसे nanocapsule, nanocapsules से nanogel और nanoemulsion। विकसित नैनोफॉर्म्यूलेशन का लक्षण वर्णन टीईएम (TEM) / डीएलएस (DLS) तथा डीएससी (DSC) द्वारा किया गया। भंडारण स्थिरता के लिए फॉर्म्यूलेशन का मूल्यांकन, भौतिक स्वरूप में परिवर्तन या भंडारण के दौरान कण आकार में परिवर्तन के आधार पर किया गया।

इसके अलावा, नैनोकैप्सूल का (आरटीयू-RTU) फॉर्म्यूलेशन मिनी कॉटन बैग पर तैयार किया गया। नैनो-माइसीलर इमल्शन का उत्पादन अपरिपक्व मच्छरों के नियंत्रण के लिए किया गया। नैनोजेल को सूती वस्त्र पर लगाया गया ताकि धुलाई के बाद भी लंबे समय तक प्रभावी एवं टिकाऊ मच्छर प्रतिरोधी कपड़े का उत्पादन किया जा सके। नैनोकैप्सूल रेशों के साथ प्रतिक्रिया या बंधन नहीं कर सकते हैं, इसलिए उन्हें फाइबर से बांधना आवश्यक है। Acrylates का उपयोग नैनोकैप्सूल को कपड़े से बांधने के लिए किया जा सकता है, जो नियमित धुलाई के बाद भी कपड़े से नहीं छूटता। काइटोसोन नैनोकैप्सूल-लेमनग्रास (*सिंबोपोगोन साइट्रेटस*) तेल (एलजीओ) युक्त को जेल के रूप में विकसित किया गया, जिसमें एक्रिलेट (एसी) को थिकनर और फैब्रिक बाइंडर के रूप में शामिल किया गया था। लंबे समय तक चलने वाली और धुलाई के बाद भी न निकलने वाली मच्छर रोधकता प्राप्त करने के लिए जेल को कपड़े पर लगाया गया। कपास के रेशों और जेल के बीच परस्पर क्रिया की जांच एफटीआईआर और एक्सआरडी द्वारा की गई। जेल के वॉश ड्यूरेबिलिटी की तुलना बिना एक्रिलेट वाले काइटोसोन नैनोकैप्सूल से SEM और GC-MS का उपयोग करके की गई। एसईएम विश्लेषणों से पता चला है कि नैनोकैप्सूल युक्त एक्रिलेट नियमित धुलाई के बाद भी कपड़े पर बरकरार रहता है। जीसी-एमएस परिणामों ने संकेत दिए कि कपड़े में कम करने योग्य तेल घटकों की मात्रा एक्रिलेट युक्त नैनोकैप्सुल्स (एलजीओ-एनकैप-एसी) में धुलाई की श्रृंखला के बाद अधिक पाई गयी, जो आगे बेहतर वॉश ड्यूरेबिलिटी और कपड़े पर कैप्सूल के प्रतिधारण की ओर संकेत करता है। पंद्रह धुलाई के बाद के जैव-प्रभावकारिता परिणाम दर्शाते हैं कि एक्रिलेट के उपयोग के साथ मच्छरों के खिलाफ 75 प्रतिशत रोधकता बनी रही, जबकि एक्रिलेट रहित नैनोकैप्सूल में यह केवल 51 प्रतिशत थी। इसके अलावा, स्विस् एल्विनो चूहों पर नैनोजेल के 36 दिनों के नियमित प्रयोगों के बाद भी त्वचीय विषाक्तता का कोई संकेत नहीं दिखा। इस प्रकार, यह फॉर्म्यूलेशन सैन्य कर्मियों और फील्ड ड्यूटी करने वाले व्यक्तियों, जहां मच्छरों के काटने का खतरा अधिक है, की पोशाक तैयार करने के लिए उपयुक्त है।

देवदार (*सेडरस देवदार*) तेल युक्त पेक्टिन नैनोकैप्सूल का उत्पादन किया गया। नैनोकैप्सूल को उनके आकारिकी, आकार, एनकैप्सुलेशन दक्षता और थर्मल स्थिरता के अनुसार चित्रित किया गया। इसके अलावा, नैनोकैप्सूल को मिनी कॉटन टी बैग्स पर लगाया गया जिन्हें मच्छरों के प्रजनन स्थलों के उपचार के लिए आरटीयू (RTU) फॉर्मूलेशन के रूप में नियोजित किया गया था। पेक्टिन-देवदार तेल युक्त नैनोकैप्सूल के साथ तैयार बैगों की लार्वासाइडल गतिविधि का मूल्यांकन मलेरिया वेक्टर, *एनोफिलीज क्युलिसिफेसिस* के खिलाफ किया गया इस दौरान 4 सप्ताह तक 98 प्रतिशत मृत्यु दर दर्ज की गई। यह मच्छर वेक्टर को नियंत्रित करने में इसके संभावित और परेशानी मुक्त अनुप्रयोगों को इंगित करता है। इसके अलावा नैनो कैप्सूल माउस सेल लाइन पर भी सुरक्षित पाए गए।

काजू (*एनाकार्डियम ऑक्सीडेंटले*) शेल लिक्विड (सीएनएसएल) काजू प्रसंस्करण उद्योग से निकलने वाला एक अपशिष्ट उप उत्पाद है। पारिस्थितिक मुद्दों के संदर्भ में सीएनएसएल(CNSL) की बड़ी मात्रा का निपटान एक चिंता का विषय बन सकता है। सीएनएसएल में कीटनाशक गुण हैं, इस तथ्य के बावजूद भी इसका पूरी तरह से उपयोग नहीं किया गया है। सीएनएसएल पानी में अघुलनशील है। इस संदर्भ में, हमने सीएनएसएल आधारित लार्वासाइडल अनुप्रयोगों हेतु नैनोइमल्शन विकसित करने के लिए स्पॉन्टेनस एमुल्सिफिकेशन विधि का उपयोग किया। इस विधि में सीएनएसएल के 52 नैनोमीटर माध्य व्यास वाले मोनोडिस्पर्स माइसेल्स का उत्पादन किया गया। थोक(bulk) और नैनो सीएनएसएल की तुलनात्मक जैव-प्रभावकारिता का मूल्यांकन *एनोफिलीज क्युलिसिफेसिस* के तीसरे इंस्टार लार्वा के खिलाफ किया गया, जिसमें क्रमशः 18.1 mg/L और 1.4 mg/L के LC50 दर्ज किया गया था। सीएनएसएल (CNSL) नैनोइमल्शन के संपर्क में आने के बाद लार्वा के अकारिकी नुकसान (morphological damage) की जांच स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोप (SEM) का उपयोग करके की गई। हिस्टोपैथोलॉजिकल परीक्षा से लार्वा पर नैनो सीएनएसएल की विषाक्तता के विस्तृत तरीके का पता चला। प्राप्त परिणाम बताते हैं कि सीएनएसएल नैनोइमल्शन ने थोक(bulk) सीएनएसएल की तुलना में लार्वासाइडल गतिविधि को बढ़ाया है, जो जैविक अपशिष्ट के प्रभावी उपयोग का अवसर देता है। सीएनएसएल नैनोइमल्शन का उत्पादन काजू उद्योग द्वारा बड़े पैमाने पर पैदा होने वाले अपशिष्ट के निष्पादन संबंधी समस्या को हल करने में किया जा सकता है। इस तरह का दृष्टिकोण मच्छर वेक्टर के खिलाफ बड़ी हुई प्रभावकारिता के लिए उपयुक्त हो सकता है। साथ ही सिंथेटिक कीटनाशकों के कम से कम उपयोग को भी बढ़ावा दिया जा सकता है।

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LIST OF ABBREVIATIONS

Syntax	Descriptions
UNDP	United National Development Program
NVBDCP	National Vector Borne Disease Control Program
WHO	World Health Organization
IRS	Indoor Residual Spray
ITN	Insecticidal Treated Net
SIT	Sterile Insect Technique
(ASTB)	Attractive sugar toxic bait
OP	Organophosphate
EO	Essential oil
Aza	<i>Azadirachtin</i>
OP	<i>Orange peel</i>
UTL	<i>Used tea leaves</i>
NOEC	Non-edible oil seed cakes
CNSL	Cashewnut Shell liquid
AI/ AIs	Active Ingredient / Active Ingredients
TPP	Tri polyphosphate
DE	Degree of esttrification
OECD	Organization for economic corporation & development
DEET	N,N-Diethyl-meta-toluamide
GSH	Reduced glutathione
GST	Glutathione S-Transferase

LPO	Lipid Peroxidation level
MDA	Malondialdehyde
LS	Longitudinal Section
LGO	Lemongrass oil
EE	Encapsulation efficiency
LGO-enap-Ac	Lemongrass encapsulated gel with Acrylate
LGO encap	Lemongrass encapsulated with out acrylate
Wo/Ac	Without acrylate
W/Ac	With Acrylate
GRAS	Generally Regarded As Safe
IHC	International Council for Harmonisation
ROS	Reactive oxygen species
CWO	Cedar wood oil
SD	Steam Distillation
RTU	Ready to use
MTT	3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide)
NCCS	National Centre for Cell Science
DMSO	Dimethylsulphoxide
M	Muscle
EC	Epithelial cells
AT	Adipose Tissue
LC ₅₀	Lethal Concentration
HLB	Hydrophilic lipophilic Balance