ORIGINAL ARTICLE

Formulation and evaluation of cashew nut shell oil and pungam oil-loaded nanocapsules for larvicidal activity against *Aedes aegypti*

Radhika Parasuram Rajam, M. Kavin Raj, Giriprasath Shanmugam

Department of Pharmaceutics, The Erode College of Pharmacy, Erode, Tamil Nadu, India

Abstract

Objectives: Mosquitoes are the potential vectors of many diseases such as dengue malaria and brain fever. Effective control of vector borne diseases is possible by early diagnosis and prompt treatment of vector control. Nanotechnology, a promising field of research in the present decade, is expected to give major impulses to technical innovation. Larviciding is a key strategy used in many vector control programs around the world. In recent years, essential oil-based larvicides have been introduced as alternatives to industrial ones. **Methods:** The present work was carried out to formulate nanocapsules containing natural oils (Cashew nut shell oil [CNSL] and pungam oil) using gum Arabic and maltodextrin as polymer which does not require complex apparatus and special precautions by emulsion diffusion/solvent evaporation method and to determine the mortality rate of the dengue larva using various concentrations of prepared nanocapsules. Physicochemical properties and duration of larvicidal action of the formulated nanocapsules were investigated. **Results:** The formulation F4 has better encapsulation efficiency (78.08 ± 0.91) when compared to other formulated nanocapsules and the particle size was found to be 714.1 nm with polydisperse index of 0.105. Zeta potential of the nanocapsule formulation was found to be around −42.0 mV which is essential for long stable formulation. The comparison of FTIR spectra of pure oil samples (CNSL: Pungam oil) and formulated nanocapsule F4 confirms the entrapment of drug in the formulation, without any chemical interaction. The scanning electron microscopy photographs reveal that the particle size of the nanocapsules were within 1000 nm. The mean particle size of the nanocapsule observed by transmission electron microscopy is comparable with the mean size obtained using the Malvern Zetasizer. In 2000 µg/mL concentration of nanocapsules, metabolite showed strong activity against *Aedes aegypti* larvae and the mortality rate was higher. The LC_{50} value of the given test samples (Pungam Oil, CNSL, and Nanocapsule Formulation F4) was found to be 1402.81 µg/mL, 270.39 µg/mL, and 1545.25 µg/mL, respectively. The stereomicroscopical study of CNSL and pungam oil nanocapsules of 2000 µg/mL concentration treated with *A. aegypti* induced toxic effects on many effects on many regions of the body. **Conclusion:** In summary, our study shows that the prepared nanocapsules present a clear larvicidal effect against third instar larvae of *A. aegypti* larvae. Therefore, the results suggest that CNSL and pungam oil has the potential to be used for the development of novel larvicides against dengue larva. The polymeric nanocapsules showed a longer duration of action and also higher efficacy.

Key words: Cashew nut shell oil, herbal drugs, larvicidal activity, nanocapsules, pungam oil

INTRODUCTION

More than half of the world's population lives in areas where several species of mosquitoes are present. Vector control is an essential requirement in control of epidemic diseases such as malaria, filariasis, and dengue that are transmitted by different species of mosquitoes. Over the last decades, climate change, population growth, deforestation, habitat invasion, and insecticide resistance have contributed to the emergence, re-emergence, and dispersion of several vector-borne diseases. Among these, those transmitted by the mosquito *Aedes* aegypti (*Diptera*: *Culicidae*), include dengue fever, yellow fever,

Address for correspondence:
Dr. Radhika Parasuram Rajam, Department of Pharmaceutics, The Erode College of Pharmacy, Erode, Tamil Nadu, India. Mobile: +91-9445257740.
E-mail: radhikannan2005@gmail.com

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chikungunya, and Zika, are some of the major challenges of public health in a vast region of our planet, affecting the lives of hundreds of millions of people every year.[1] *A. aegypti* is a mosquito species that can carry and transmit the dengue virus causes dengue hemorrhagic fever, yellow fever virus, and chikungunya virus.

The use of synthetic compounds can lead to increased mosquito resistance, environmental pollution caused by their residue, and the death of non-target creatures. Botanical biocides have been used for over 3000 years[2] which can be incorporated in pest management for commercial crops, including organic production systems.[3] The advantages of the use of botanical biocides are rapid degradation by sunlight, low persistence in the environment, lower likelihood of the target organism developing resistance, and low residual activity. The natural biopesticides are usually easy to apply and not harmful to natural enemies and other beneficial insects. No vaccine is currently commercially available against the viral diseases transmitted by *A. aegypti*. Therefore, the prevention of these diseases is mainly achieved through mosquito population control and the development of new biodegradable, eco-friendly, and specific larvicides is of paramount importance for future control strategies.[4]

The best way to keep the mosquitoes at check is larviciding. Therefore, the use of plants as a bio pesticide is a one of alternative natural way for controlling in a safer environment balance. Larvicides derived from botanical extracts constitute a new and promising category of pesticide, due to their reduced toxicity for non-target species and low environmental pollution. Unlike conventional insecticides, which normally contain one specific active agent, plant-derived larvicides usually contain a combination of several chemical compounds that work synergistically, targeting different biological processes.[5] One-way to overcome such limitations is to use the types of carrier systems first developed for health applications and widely used in association with drugs, which show promise for reducing the use of pesticides as well as the problems caused by them. The modified release characteristics of these systems have attracted the attention of researchers for the development of formulations that can be loaded with active agents used in agriculture, such as botanical biocides.

Nanotechnology has a wide application in vector control in the form of nanocapsules for herbicide delivery and vector and pest management and nanosensors for pest detection. Polymer-based nanocapsules have been widely studied as a potential drug delivery system in recent years. The advantages of polymeric nanoparticles include biocompatibility, biodegradability, the ability to modify and functionalize the surface, incorporation of the active agent without any chemical reactions, and the possibility of modulating the degradation. In the recent year's, natural oil-based nanoformulations have been introduced as an alternatives to industrial ones or synthetic formulation. To overcome the mentioned problem, encapsulation of natural active agents has been proposed. Applications of nanotechnology have been extended in the field of mosquito control by the nanocapsules from environmentally acceptable plant extract. Synthesized nanocapsules help to produce new insecticides and insect-repellants.[6]

Keeping an unpolluted and hazardless environment in mind, a number of plant products have been evaluated against mosquito larvae. In the recent years, natural oil-based nanoformulations have been introduced as an alternatives to industrial ones or synthetic formulation. Controlling the mosquito larvae, especially in the endemic region, are probably the easiest and the best cost effective way than to control mosquito borne disease and their transmission. However, occurring resistance in mosquito environmental pollution and adverse effects on non-targeted species have been observed continuously due to the synthetic larvicides. To overcome the mentioned problem, encapsulations of natural active agents have been proposed. The main disadvantage of the plant derived oils is their very short shelf-life.[7]

The pungam tree oil and cashew nut shell oil stands out as a species used by various populations for insecticidal purposes, such as the elimination of fleas, lice, flies, and other insects. The oil is an important constituent of the nanocapsules, due to its ability to retain and solubilize lipophilic drugs.[8-11] The concentration and nature of the oil influences the mean particle size and particle size distribution,[12-17] which, in turn, influence drug release kinetics.[18] For this reason, it is important to appropriately choose the oily constituent when developing a new carrier system, especially when the goal is to load a lipophilic substance into the nanocarriers, which is the major application of nanoparticles.

Among them, cashew nut shell liquid and Pongamia pinnata have the larvicidal activity due to the Cardanol and Karanjachrome present, respectively, against *A. aegypti* mosquitoes. Nanocapsules containing cashew nut shell oil and pungam oil with the polymers gum Arabic and maltodextrin were formulated using various concentrations to investigate its larvicidal activity against dengue larvae (*A. aegypti*).

MATERIALS AND METHOD OF PREPARATION

Materials Required

Cashew nut shell oil was obtained as a gift sample from Kavyashree oil mills, Erode, Pungam oil obtained as a gift sample from Pragathi oil mills, Erode, gum arabic, SRS Pharmaceutical's Pvt Ltd, Mumbai.,

Phytochemical Tests[19]

The cashew nut shell oil and pungam oil were subjected to various phytochemical screening test such as flavonoids,

alkaloids, tannins, saponins, glycosides, terpenoids, reducing sugars, volatile oils, proteins, amino acids, and phytosteroids as per the standard procedure to reveal the presence of various plant constituents present.

Preparation of Cashew Nut Shell Oil (CNSL) and Pungam Oil Nanocapsules[20]

Prepared gum Arabic (0.25 g) and maltodextrin (0.25 g) solutions were mixed together. These solutions were placed under the magnetic stirrer at 500 rpm under 50°C until the clear solution was formed. Then add 10 mL of 1% Tween 80 to the clear solution in drop wise manner under constant stirring. Finally, the CNSL and pungam oil was taken and dissolved completely in ethanol and was added drop wise to the above solution until the nanocapsules were formed. The prepared Nanocapsules were finally spray dried and stored in the airtight container. Nanocapsules with different concentration of drug and polymer (F1-F5) were prepared according to Table 1.

Characterization of the Nanocapsules

The formation and completion of the nanocapsules were characterized by double-beam UV spectrophotometer (Systronics, Mumbai)[21] FTIR spectroscopy was used to investigate and predict any physiochemical interactions between selected polymers maltodextrin, gum arabic and the drugs pungam oil, and CNSL and to identify the compatibility between physical mixture of drug, and polymer and drug-loaded nanocapsules.[22] The spectra obtained for these samples were compared and interpreted for the shifting of major functional peaks and disappearance of functional peaks if any. The size of the nanoparticles was determined by Zetasizer (Nano ZS, Malvern instruments, Malvern, UK) based on dynamic light scattering technique.[23] The polydispersity index, which is a dimensionless number indicating the width of the size distribution, was also measured.

Drug Entrapment Efficiency[24]

The total amount of the drug and the polymer in the nanocapsules was analyzed by dissolving 20 mg of sample in 5 mL of chloroform after the polymer was dissolved, 20 mL of water was added, and mixture was mixed carefully in a separating funnel. The amount of drug in the water phase was detected by UV-Spectrophotometer at 272 nm. The entrapment efficiency (%) of drug was calculated by following equation.

$$\%\ Entrapment\ efficiency = \frac{Mass\ of\ drug\ in\ Nano\ capsules}{Mass\ of\ drug\ used\ in\ formulation} \times 100$$

Table 1: Composition of CNSL and pungam oil nanocapsules

Formulation code	Gum Arabic (mg)	Maltodextrin (mg)	CNSL and Pungam oil (mg)
F1	500	250	200
F2	750	250	250
F3	750	500	400
F4	250	250	300

CNSL: Cashew nut shell oil

Scanning Electron Microscopy (SEM)[25]

SEM (JEOL 5400, TOKYO, JAPAN) was used to decide the shape, surface topography, and texture as well as to inspect the morphology of cracked or sectioned surface. The average particle size of nanocapsules was determined by measuring the diameter of all the capsules and the magnification factor was calculated using the scanning micrograph. The ultrastructure of the nanocapsules was investigated using transmission electron microscopy (TEM), used for assessing the particle size distribution inside the skin. The analyses were performed using JEM-1400 TEM (JEOL, Peabody, MA, USA), operated at 100 kV, and captured on advanced microscopy techniques (AMT) 1K CCD, using the software AMTV602 (AMT, Woburn, MA, USA).

Larvicidal Bioassay

The larvae of the mosquito species *A. aegypti* were collected from the aquatic environment of Chennai District. Biscuits were served as larval food. The larvae were kept at 25 ± 2°C and proper photoperiod was given for their growth. Late third and early fourth instars, larvae were (4–5 mm in length) used for larval bioassay purpose. The morphological and anatomical characteristics of the collected larvae were observed and identified through microscopic analysis and compared to the standard keys.

Larvicidal bioassay was performed based on the World Health Organization, (2009) protocol. *A. aegypti* mosquito larvae were exposed to a wide range of concentration of crude extracts, that is, 125, 250, 500, 1000, and 2000 µg/mL and control to find out activity. Batches of 25 healthy fourth instar larvae were transferred to the 250 mL water containing chambers and different concentrations of test samples were added to assess the desired target dosage, equal number of controls was also setup with tap water. Larval mortality was observed after 24 h. Mortality percentage was calculated using formula.

$$Mortality\ (\%) = \frac{X - Y}{X} \times 100$$

Where, X= survival in the untreated control. Y= survival in treated sample.

Histopathological and Stereomicroscopic Analysis[26]

Thin section of *A. aegypti* larval tissues treated with nanocapsules were prepared (8 µm thickness) using microtome. Using a clean glass slide, the sectioned tissues were mounted and stained using hematoxylin and eosin stain. The effects of larval toxicity of extract were observed under bright field light microscope at ×40. The extract accumulation of tissue and its alterations were seen under a stereomicroscope.

Table 2: Encapsulation efficiency of different formulation

S. No.	Batch code	Drug entrapment efficiency (%)
1.	F1	70.67±0.90
2.	F2	72.08±0.87
3.	F3	60.12±0.92
4.	F4	78.08±0.91

RESULTS AND DISCUSSION

Phytochemical Test Observation

Phytochemical analysis of CNSL and pungam oil showed a variety of rich secondary metabolites such as alkaloids,

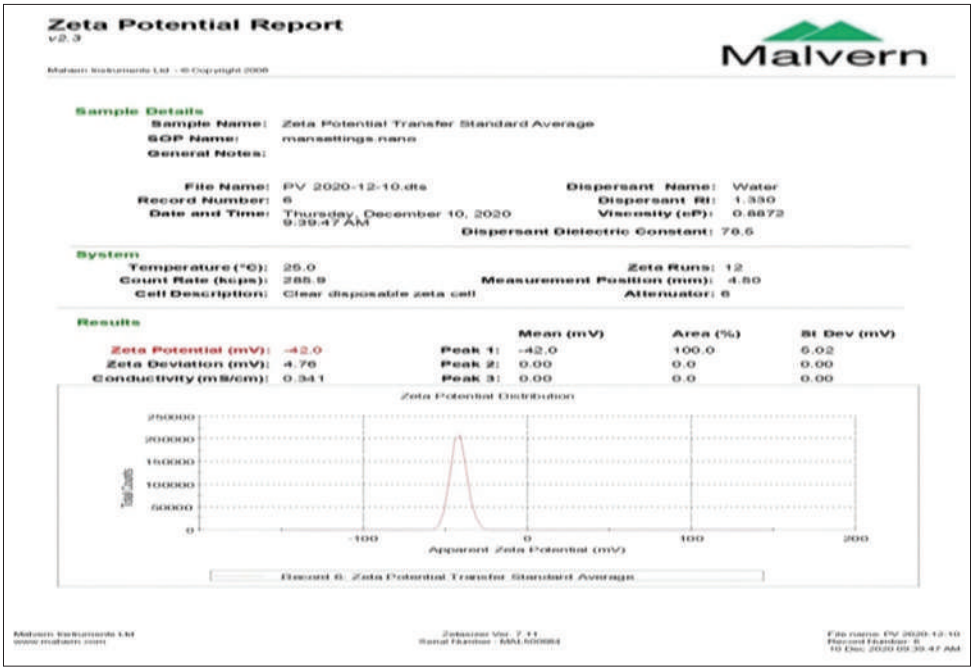

Figure 1: Zeta potential report of formulated nanocapsule F4

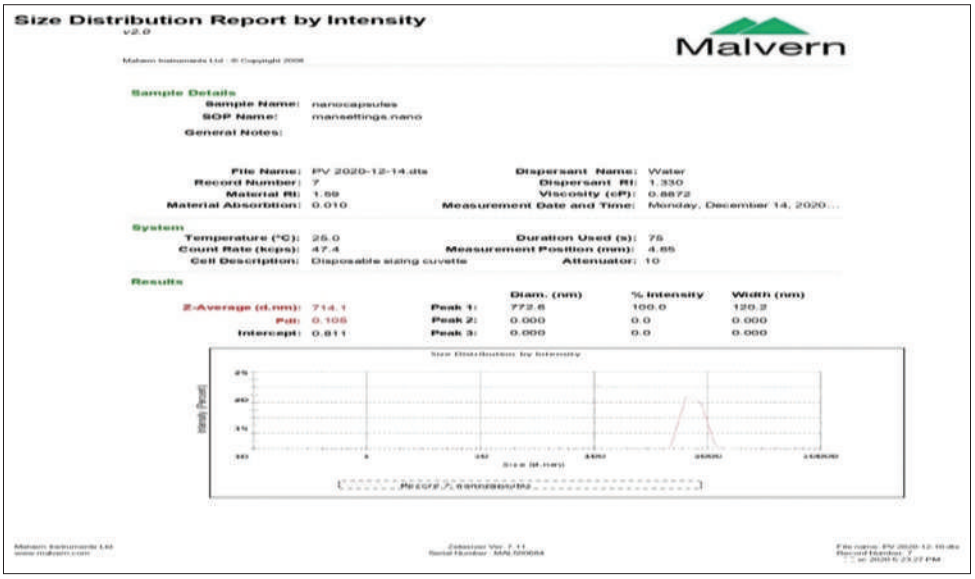

Figure 2: Size distributions by intensity of formulated nanocapsule F4

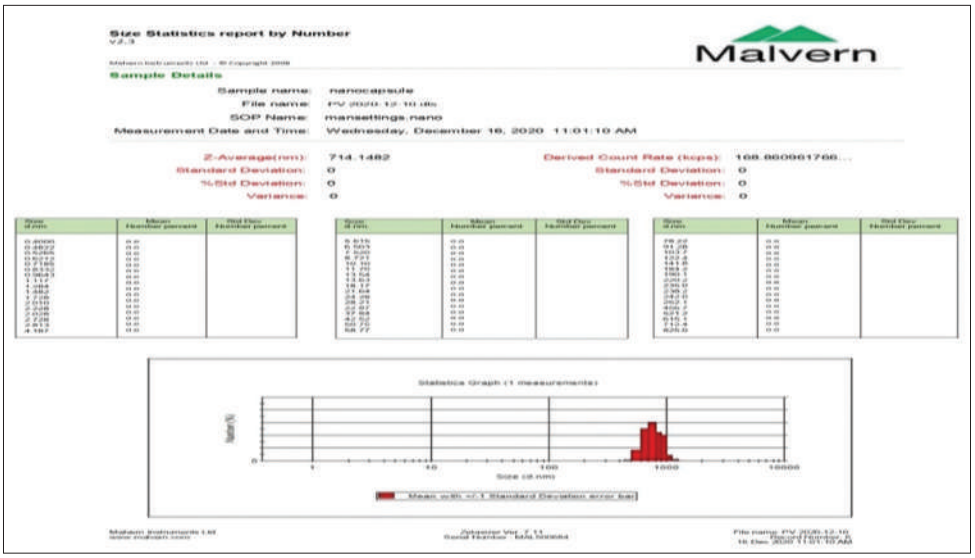

Figure 3: Size statistics report by number of formulation F4

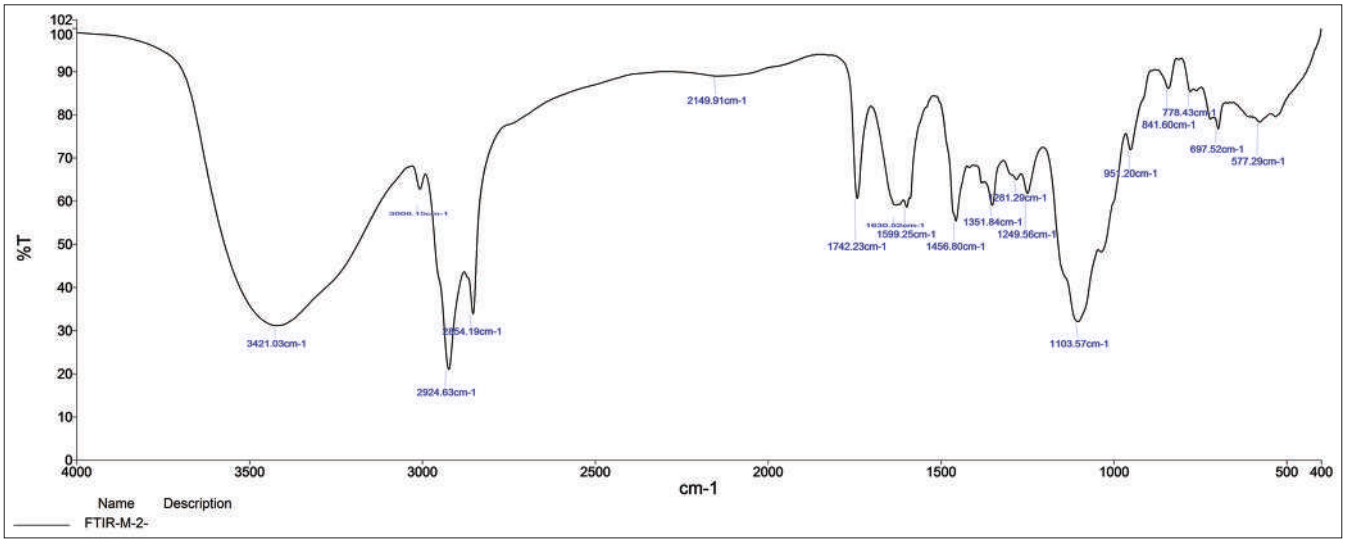

Figure 4: FT-IR spectra of nanocapsule formulation F4

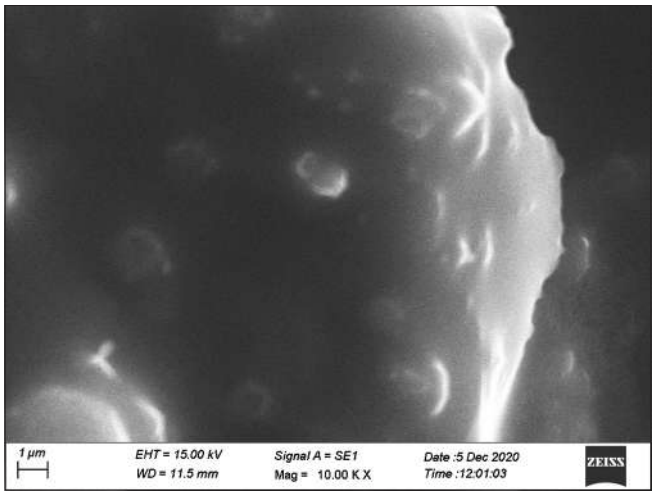

Figure 5: Scanning electron microscopy image of formulated nanocapsule F4

saponins, phenols, tannins, flavonoids, amino acids, terpenoids, proteins, steroids, and glycosides. Acetone was found to be effective in dissolving the phytochemicals. The presence of phenolic lipids confirms the presence of anacardic acid which has an increased larvicidal activity against various mosquito species. The presence of high amount of flavonoids confirms the presence of karanjin which has an increased larvicidal activity against various mosquito species.

Characterization of Formulated Nanocapsules with Cashew Nut Oil and Pungam Oil

The UV spectrum of the prepared nanocapsules formulation with cashew nut oil and pungam oil was identified and the maximum wavelength of the prepared nanocapsules was found to be at 272.45 nm.

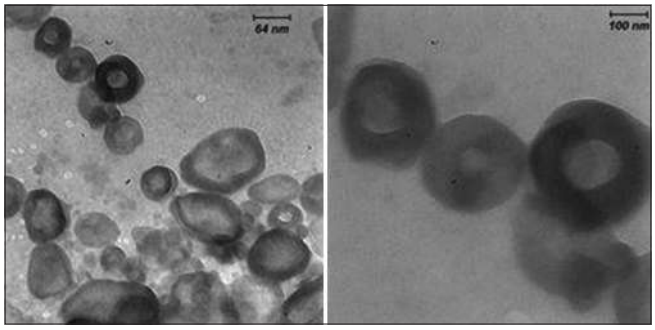

Figure 6: Transmission electron microscopy images of nanocapsules

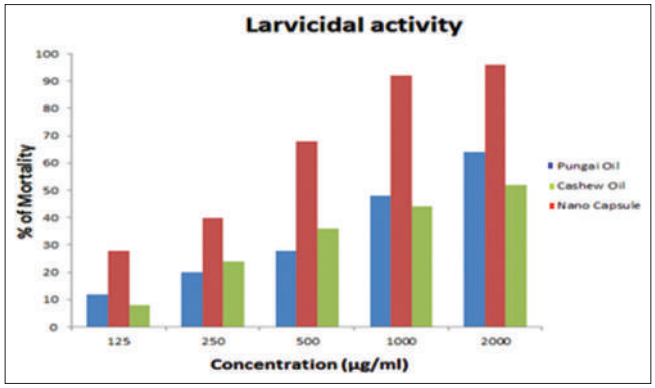

Figure 7: Schematic representation of the mortality against concentration of individual oils and nanocapsule formulation F4

Drug Entrapment Efficiency

The effect of initial CNSL and pungam oil drug loading entrapment efficiency for gum arabic and maltodextrin nanocapsules was in the range between 60.12 ± 0.92% and 78.08 ± 0.91% [Table 2]. The formulation F4 has better encapsulation efficiency when compared to other formulated nanocapsules.

Zeta Potential, Particle Size Analysis, and Size Statistics Report by Number of the Formulated Nanocapsule (F4)

The size of the nanocapsule formulation F4 was confirmed by Zetasizer and the particle size was found to be 714.1 nm and polydisperse index was found to be 0.105 [Figures 1-3]. Zeta potential is an important parameter to analyze the long-term stability of the nanocapsules. Zeta potential of the nanocapsule formulation was found to be around −42.0 mV which is essential for long stable formulation.

FT-IR Spectra of Nanocapsule Formulation (F4) containing CNSL and Pungam oil

The chemical interaction between the drug and polymer and other excipients was studied using FTIR [Figure 4]. The comparison of FTIR spectra of pure oil samples

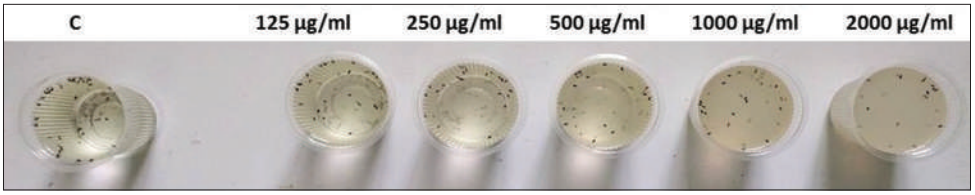

Figure 8: Schematic representation of the mortality of dengue larva against different concentrations of cashew nut shell oil

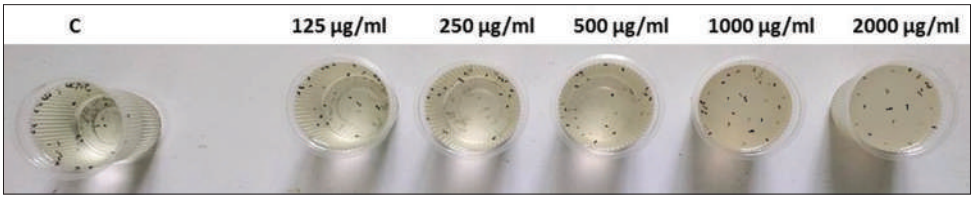

Figure 9: Schematic representation of mortality of dengue larva against different concentrations of Pungam oil

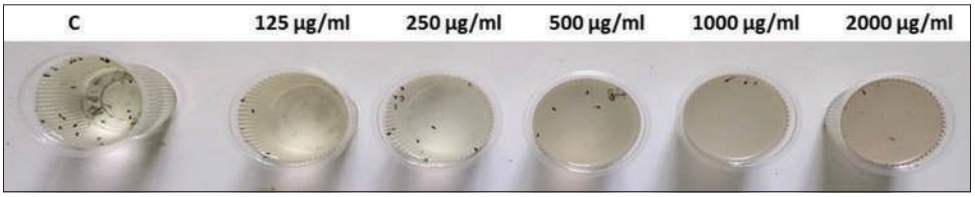

Figure 10: Schematic representation of the mortality of dengue larva against different concentrations of prepared nanocapsule formulation F4. *C-control

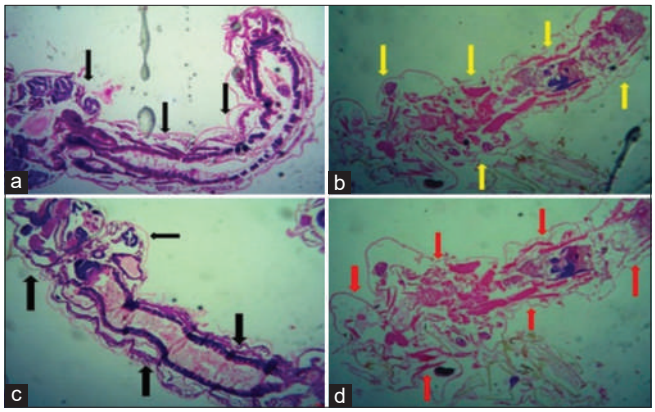

Figure 11: (a-d) Histopathological study on treated third instar larvae of *Aedes aegypti* with nanocapsules. Black arrow represents control larvae structures, yellow and red arrow indicates treated larval structure

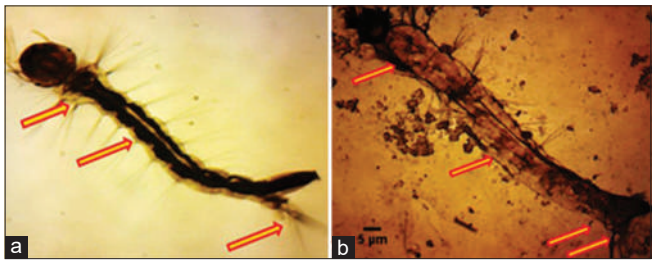

Figure 12: (a) Represent stereomicroscopic image of untreated larva. (b) Image after treatment with cashew nut shell oil and pungam oil nanocapsules

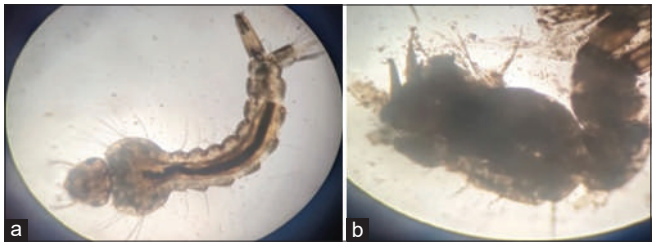

Figure 13: Represents microscopic image of untreated larva and image after treatment with cashew nut shell oil and pungam oil nanocapsules

(CNSL: Pungam oil) and formulated nanocapsule F4 further confirms the entrapment of drug in the formulation, without any chemical interaction. From the FTIR studies, it was concluded that the selected polymer maltodextrin and gum Arabic was found to be compatible in entrapping the cashew nut shell oil and pungam oil.

SEM

The surface characteristics and the particle size of the cashew nut shell oil and pungam oil nanocapsules were studied by SEM. The SEM photographs reveal that the particle size and shape of the nanocapsules were within 1000 nm [Figure 5].

TEM

Size of the nanocapsules was observed by transmission electron microscope (TEM) [Figure 6]. Spherical nanocapsules with homogenous size were formed, with no agglomerations can be seen and the shell surrounding the oily core was limited by an external polymeric layer (Maltodextrin and gum arabic) with surfactants (Tween 80) as stabilizers. The capsular shape and structure show the polymer membrane as a line surrounding an oily core of cashew nut shell oil and pungam oil. In all the formulations, the nanocapsules presented spherical morphology with diameters between 650 and 800 nm. The mean particle size of the nanocapsule observed by TEM is comparable with the mean size obtained using the Malvern Zetasizer.

Larvicidal Bioassay

After treatment of CNSL and pungam oil nanocapsules, significant mortality of larvae against *A. aegypti* was recorded and larvae suffered with severe deformities. The sublethal effects on fourth instars larval were also found on early instars. Third instar larvae were highly susceptible at the optimized concentrations. In 2000 µg/mL concentration of nanocapsules, metabolite showed strong activity against *A. aegypti* larvae and the mortality rate was higher [Figures 7-10].

The LC_{50} value of the given test samples (Pungam oil, CNSL, and nanocapsule formulation F4) was found to be 1402.81 µg/mL, 270.39 µg/mL, and 1545.25 µg/mL, respectively.

Mechanism of Action of Larvicidal Nanocapsules

The presence of flavonoids such as karanjin and karajachrome in pungam oil acts as a strong inhibitor of ache from mosquito larva indicating it to the presumable site of larvicidal action. It has also been observed that the karanjin flavonoid can inhibit feed and act as a growth regulator. The same results were observed in testing *A. aegypti* by anacardiac acid and caused histomorphological changes in the midgut. The cashew nut shell oil contains trace amounts of alkaloids which have an influence in the central nervous system of the larva, acting on the receptors of several neurotransmitters, provoking uncontrolled muscular movements, paralysis, seizures, and death.

Histopathological and Stereomicroscopic Analysis

The histopathological study of nanocapsules formulated with CNSL and pungam oil treated with *A. aegypti* larvae showed collapse and broken epithelial cell layers and mosquitoes showed an entire damage in the mid-gut and caeca areas and finally, larval structure was fully collapsed

[Figure 11] Moreover, a huge shrink age was observed in the abdominal region of *A. aegypti* larvae treated with nanocapsules (F4).

Stereomicroscopic Analysis

The stereomicroscopical study of CNSL and pungam oil nanocapsules of 2000 µg/mL concentration treated with *A. aegypti* induced toxic effects on many effects on many regions of the body including thorax, abdomen, and anal gill (red arrows) indicates loss of external hairs, crumbled epithelial layer of the outer cuticle and shrinkage of the larvae, respectively, Figures 12 and 13.

CONCLUSION

It is known that botanical insecticides are useful for mosquito control due to a wealth of secondary metabolites, which are applied when the evolution and adaptation suffered against predators. Herbal larvicides have the advantage of eliminating a large population of individuals in a small area, being a good alternative in eliminating vectors. The use of natural products as larvicides is highly preferred than chemical larvicides, due to their rapid environmental degradation and no toxicity to other non-target organisms.

Overall, this study strengthens the idea that the use of natural products associated with nano systems and can be an excellent alternative in the search for new, more selective, and biodegradable compounds for the control of *A. aegypti* larvae, and a promising alternative in the face of the increase in resistance against the traditionally synthetically chemicals used for this purpose. Cashew nut oil and pungam oil was an effective larvicide against *A. aegypti* larvae that it was highly toxic to mosquito larvae and inhibited the development of pupae.

In summary, our study shows that the prepared nanocapsules present a clear larvicidal effect against third instar larvae of *A. aegypti* larvae. Therefore, the results suggest that CNSL and pungam oil has the potential to be used for the development of novel larvicides against dengue larva. The polymeric nanocapsules showed a longer duration of action and also higher efficacy. It is obvious that the performance of combined application of CNSL and pungam oil has better action against the dengue larva than their individual application. Although the individuals are good against the mosquito larva, the synergistic effect is well exhibited.

This study demonstrates the potential of this herbal nanoformulation as a suitable alternative for synthetic larvicides in terms of long-lasting activity, efficiency, and environmental friendliness, which makes the society aware of utilization of green waste in scientific manner.

Further research needs to be done about the toxicity effects of a mixture of CNSL and pungam oil against non-target organisms, that is, fish and frog and to evaluate other mechanism of actions at the molecular level to determine the site of action on DNA and how the splicing occurs in the cellular level.

REFERENCES

1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, *et al*. The global distribution and burden of dengue. Nature 2013;496:504-7.
2. Morgan ED. Azadirachtin, a scientific gold mine. Bioorg Med Chem 2009;17:4096-105.
3. Keita SM, Vincent C, Schmit JP, Arnason JT, Bélanger A. Efficacy of essential oil of *Ocimum basilicum* L and *O. gratissmum* L, applied as an insecticidal fumigant and powder to control *Callosobruchus maculatus* (Fab.). J Stored Prod Res 2001;37:339-49.
4. Hurst TP, Kay BH, Ryan PA, Brown MD. Sublethal effects of mosquito larvicides on swimming performance of larvivorous fish *Melanotaenia duboulayi* (*Atheriniformes*: *Melanotaeniidae*). J Econ Entomol 2007;100:61-5.
5. Thiyagarajan P, Kumar PM, Kovendan K, Murugan K. Effect of medicinal plant and microbial insecticides for the sustainable mosquito vector control. Acta Biologica Indica 2014;3:527-35.
6. Adhikari U, Ghosh A, Chandra G. Nano particles of herbal origin: A recent eco-friend trend in mosquito control. Asian Pac J Trop Dis 2013;3:167-8.
7. Richards DA, Maruani A, Chudasama V. Antibody fragments as nanoparticle targeting ligands: A step in the right direction. Chem Sci 2017;8:63-77.
8. Nagavarma BV, Yadav H, Hemant KS, Ayaz A, Vasudha LS, Shivakumar HG. Different techniques for preparation of polymeric nanoparticles a review. Asian J Pharm Clin Res 2012;5:16-23.
9. Shastri VP. Non-degradable biocompatible polymers in medicine: Past, present and future. Curr Pharm Biotechnol 2003;4:331-7.
10. Farrugia CA, Grover MJ. Gelatin behavior in dilute. In: Kreuter J, editor. Nanoparticles. Colloidal Drug Aqueous Solutions: Designing a Nano Particulate Formulation, Delivery Systems. New York: Marcel Dekker; 1994. p. 643-9.
11. Ghosh PK. Hydrophilic polymeric nanoparticles as drug carriers. Indian J Biochem Biophys 2000;37:273-82.
12. Boyer C, Whittake MR, Bulmus V, Liu J, Davis TP. The design and utility of polymer-stabilized iron-oxide nanoparticles for nanomedicine applications. NPG Asia Mater 2010;2:23-30.
13. Younes I, Rinaudo M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. Mar Drugs 2015;13:1133-74.
14. Wang T, Feng Z, He N, Wang Z, Li S, Guo Y, *et al*.

A novel preparation of nanocapsules from alginate-oligochitosan. J Nanosci Nanotechnol 2007;7:4571-4.
15. Dhaneshwar SS, Mini K, Gairola N, Kadam SS. Dextran: A promising macromolecular drug carrier. Indian J Pharm Sci 2006;68:705.
16. Rosa P, Friedrich ML, Dos Santos J, Librelotto DR, Maurer LH, Emanuelli T, *et al*. Desonide nanoencapsulation with acai oil as oil core: Physicochemical characterization, photostability study and *in vitro* phototoxicity evaluation. J Photochem Photobiol B 2019;199:111606.
17. Dubey V, Mohan P, Dangi JS, Kesavan K. Brinzolamide loaded chitosan-pectin mucoadhesive nanocapsules for management of glaucoma: Formulation, characterization and pharmacodynamic study. Int J Biol Macromol 2020;152:1224-32.
18. Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C. Nanocapsule technology: A review. Crit Rev Ther Drug Carrier Syst 2002;19:99-134.
19. Mustapha AA, Owuna G, Ogaji JO, Ishaq UI, Idris MM. Phytochemical screening and inhibitory activities of *Anacardium occidentale* leave extracts against some clinically important bacterial isolates. Int J Pharmacogn Phytochem Res 2015;7:365-9.
20. de Góes Carneiro AP, de Aguiar AL, de Silva RB, Richter AR, de Sousa PH, de Silva LM, *et al*. Acerola by-product as a renewable source of bioactive compounds: Arabic gum and maltodextrinnanocapsules. Food Sci Technol 2020;40:466-74.
21. Hosseinzadeh H, Atyabi F, Dinarvand R, Ostad SN. Chitosan-Pluronic nanoparticles as oral delivery of anticancer gemcitabine: Preparation and *in vitro* study. Int J Nano Med 2012;7:1851-63.
22. Nair LD, Sar SK, Arora A, Mahapatra D. Fourier transform infrared spectroscopy analysis of few medicinal plants of Chhattisgarh, India. J Adv Pharm Educ Res 2013;3:196-200.
23. Kala S, Sogan N, Naik SN, Agarwal A, Kumar J. Impregnation of pectin-cedarwood essential oil nanocapsules onto mini cotton bag improves larvicidal performances. Sci Rep 2020;10:14107.
24. Akdeniz B, Sumnu G, Sahin S. The effects of maltodextrin and gum Arabic on encapsulation of onion skin phenolic compounds. Chem Eng Trans 2017;57:1891-6.
25. Quintanar-Guerrero D, Allémann E, Doelker E, Fessi H. Preparation and characterization of nanocapsules from preformed polymers by a new process based on emulsification-diffusion technique. Pharm Res 1998;15:1056-62.
26. Shahzad K, Manzoor F. Nanoformulations and their mode of action in insects: A review of biological interactions. Drug Chem Toxicol 2021;44:1-11.

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