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Environmental benignity of a pesticide in soft colloidal hydrodispersive nanometric form with improved toxic precision towards the target organisms than non-target organisms

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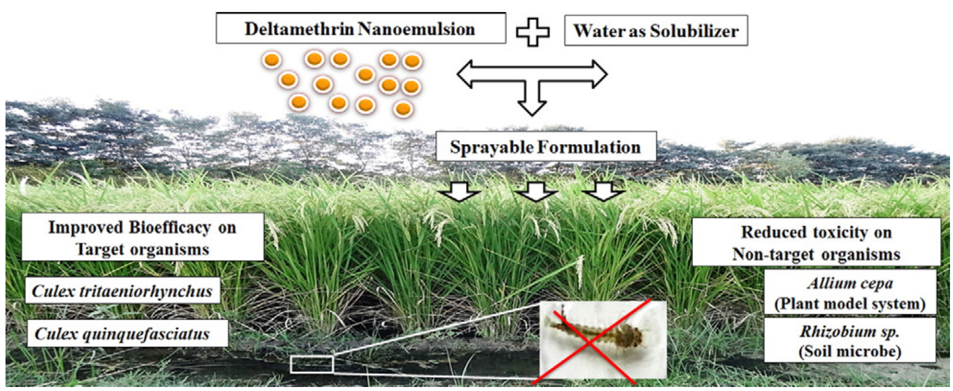
HIGHLIGHTS

- Nanopesticide research is a prerequisite for controlling the mosquito population.
- Transformation of PDM into hydrodispersive NDM by ultrasonic emulsification method.
- Improved efficacy of NDM on mosquitoes (target organisms), as comparative to PDM.
- Reduced toxic influence of NDM on *A. cepa* and RB (non-target organisms) than PDM.
- NDM possessing improved target specificity and warrants environmental benignity.

GRAPHICAL ABSTRACT



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ABSTRACT

Mosquito-borne diseases are of major concern as they cause devastating health effects, morbidity, and mortality in the human population. Conventional pesticides have failed to curb the mosquito population due to the development of insensitivity in mosquitoes. Hence, higher dosages of pesticides along with their toxic solubilizers have been employed, which have led to raise in pesticide pollution load, environmental toxicity, and human health concerns. As a realisation for the requirement of alternative pesticides, the present study has involved in the formulation of a hydrodispersive nanometric colloidal form of deltamethrin (NDM), a type-II pyrethroid pesticide, from its hydroimmisicible parental form (PDM). The mean hydrodynamic diameter of the droplets was found to be 30.6 ± 4.6 nm by dynamic light scattering study (DLS). High-resolution transmission electron micrographs have revealed the spherical structure of the droplets with a size range of 35–40 nm. The NDM was found to possess sedimentation resistance, intrinsic and hydrodispersive stability. The toxicity of NDM and PDM was comparatively investigated on target organisms (*Culex tritaeniorhynchus* and *Culex quinquefasciatus* mosquitoes) and non-target organisms (*Allium cepa* – Bioindicator of toxicants and *Rhizobium* sp. – Soil bacteria). As comparative to PDM, NDM has exerted higher efficacy on adult mosquito and larval population, even at low-level concentrations. However, in the case of non-target organisms, the NDM toxicity was lower than PDM. Comprehensively, the study has concluded the potential advantage of formulating conventional pesticides into nanometric soft

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colloidal form for the improved toxic precision on target organisms (mosquitoes). This ensures the ability of NDM to combat against the mosquito population even at lower concentrations, thereby reducing the pesticide exposure load towards the environment and human population.

1. Introduction

Mosquitoes act as a vector for several dreadful diseases such as Malaria, Dengue, Yellow Fever, Japanese encephalitis, Chikungunya, West Nile virus, Lymphatic filariasis and so on (WHO, 1996). *Culex tritaeniorhynchus* and *Culex quinquefasciatus* mosquitoes are the principal vectors of Japanese encephalitis and Lymphatic filariasis diseases respectively. The predominant dwelling and proliferation of these mosquitoes near the human habitats have raised the disease endemicity and human mortality (Bank, 1976).

The advent of synthetic pesticides such as Dichlorodiphenyltrichloroethane (DDT) has served the purpose of controlling the mosquito population since World War II. However, in later years DDT was banned due to its deliberating effect on human health and environment (Curtis and Lines, 2000). As a substitute to DDT, deltamethrin ($C_{22}H_{19}Br_2NO_3$), a member of Class II pyrethroid pesticide family has found its reliable application in mosquito control programs (Chareonviriyaphap et al., 2004). The efficacy of deltamethrin was found against a wide range of arthropods by causing membrane depolarization accompanied by the suppression of action potential amplitude (Laufer et al., 1984).

The continual and prolonged exposure of the conventional pesticides towards the mosquito population has led to the development of several adaptive resistance mechanisms to obstruct the pesticide efficacy (Hemingway and Ranson, 2000). As to overcome this, a higher dosage of pesticides was repeatedly employed in the environment. This has elevated the pesticide pollution load and toxic exposure in the environment, thereby causing acute and chronic pesticide poisoning in humans (Damalas and Eleftherohorinos, 2011). In addition, the requirement of a larger volume of toxic solvents to solubilize the hydroimmisicible pesticides has raised the pesticide and solvent associated ecological toxicity (Zhu et al., 2014). These have emphasized the need for alternative pesticides to exert higher toxicity on the targeted organism with the assurance of human health and environmental safety. The advancements in emulsion technology have provided the methods for formulating hydrophobic active compounds into nanometric colloidal dispersions called nanoemulsions. The nanoemulsion system apparently increases the availability, stability and dissolution rate of the hydrophobic active compounds (Maali and Mosavian, 2013).

By taking accord of this, the present study has formulated the hydrodispersive deltamethrin nanoemulsion (NDM) from its hydroimmisicible parental form (PDM). The methodology employed is a simple ultrasonic emulsification process to obtain the narrow size distribution of droplets. The intrinsic stability, hydrodispersive stability and the centrifugal-sedimentation resistance of NDM was investigated by DLS technique. Further, the toxicological profile of NDM and PDM was comparatively investigated on the target organisms (*Culex quinquefasciatus* and *Culex tritaeniorhynchus* mosquito population) and non-target organisms (*Allium cepa* - Plant bioindicator of toxicants and *Rhizobium* sp. - Soil microbe). The present study is first of its kind, which emphasizes the potential advantage of transforming the parental commercial pesticides into nanometric form to exert improved toxic precision towards the target organisms with the warrant of reduced toxicity towards the non-target organisms.

2. Materials and methods

2.1. Materials

The technical grade deltamethrin (PDM) with the assay purity of 98.84% was obtained from Tagros India Pvt. Ltd., which is a cream colour hydroimmisicible powder. The tween 80 (BioXtra) and analytical standard deltamethrin were obtained from Sigma-Aldrich, India. The HPLC grade solvents such as methanol, acetonitrile, water, toluene, and dimethylsulfoxide (DMSO) were procured from Merck, India. The acetocarmine stain, nutrient agar, nutrient broth, and phosphate buffer saline was obtained from HiMedia, India. The ultrapure deionized water (Milli-Q) of 18.2 MΩ cm and distilled water was obtained from Bio-water purification system (PALL Cascada, USA). The WHO polyvinyl chloride (PVC) cone (WHO cone) was obtained from Zonal Entomological Team, Vellore, Tamil Nadu, India.

2.2. Formulation of deltamethrin nanoemulsion (NDM)

500 mg of PDM powder was solubilized in 20% (v/v) of toluene and constituted as an organic phase. Subsequently, 18.82% (v/v) of tween 80 was added to 61.18% (v/v) of Milli-Q to constitute the aqueous phase. The organic phase was then added to an aqueous phase under the stirring condition (30 min). This spontaneously emulsifies the system and turns milky white. The obtained coarse emulsion was further subjected to ultrasonic emulsification (750 W-Ultrasonics, USA) for 15 min. This breaks down the conventional droplets into smaller ones and turns the system translucent.

2.3. Dynamic light scattering studies (DLS)

The mean hydrodynamic diameter (*Z*-average) and polydispersity index (PDI) of NDM droplets were assessed by 90 Plus Particle Size Analyser (Brookhaven Instruments Corporation, USA). The size of NDM droplets was determined from the intensity time fluctuation of a laser beam (670 nm) scattered at a fixed scattering angle of 90° at 25 °C.

2.4. Stability studies

The intrinsic stability of NDM droplets was determined by storing at room temperature (28 ± 3 °C) for 15 days, and assessing its *Z*-average by DLS. Further, the droplets were subjected to hydrodispersion stability and centrifugal-sedimentation resistance studies. The hydrodispersion stability was carried out by dispersing the constant aliquots of NDM in varying water dilution ratios of 1:1 (D1), 1:10 (D2), 1:100 (D3) and 1:1000 (D4). Centrifugal-sedimentation resistance of the droplets was investigated by subjecting the aliquots of NDM to the centrifugal force of 100 (S1), 500 (S2), 1000 (S3) and 2000 (S4) rpm for 10 min. Further, D1, D2, D3, D4 and S1, S2, S3, S4 were stored for 24 h, and the Z-average and PDI were determined by DLS. The storage period of 24 h was chosen, as the NDM interaction in the toxicity studies (mentioned in the below sections) were at the same time. Moreover, the influence of stress conditions on the emulsion droplets was more certainly inferred upon 24 h incubation period, as the emulsion instability phenomenon such as coalescence and Ostwald ripening occurs at a slower rate.

2.5. High-resolution transmission electron microscopy (HR-TEM)

A drop of NDM was coated onto a copper grid and air-dried in the sterile environment. The droplets morphology and size was determined by FEI TECNAI G^2 Model T-30- HR-TEM, USA.

2.6. High pressure liquid chromatography (HPLC)

The active compound (deltamethrin) concentration present in NDM was determined by Reverse phase-HPLC LAChromElite, Hitachi, Japan.