Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10–1000 nm. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Controlled release and particle degradation characteristics can readily be modulated by the choice of matrix constituents. Efficacy of *Chrysosporium tropicum*, a pathogenic fungus mediated silver and gold nanoparticles was reported against *Aedes aegypti*. The silver nanoparticles, synthesized with the leaf extract of *Eclipta prostrata*, were studied for their larvicidal activity against *Culex quinquefasciatus* and *Anopheles subpictus*. Santhoshkumar *et al* also synthesized silver nanoparticles using *Nelumbo nucifera* leaf extract and studied their larvicidal activity against filariasis and malaria vectors.

A number of liposomes (lipidic nanoparticles) currently in the market are the first nanomedicine delivery system to make the transition from concept to clinical application, and these are now on the established technology platform with considerable clinical acceptance. Until recently, there has been no report on the development of nanoparticles for the used chemical larvicides in the mosquito control programme.

The current study deals with the preparation of nanoparticles of two mosquito larvicides of organophosphorous group, viz. temephos and pirimiphos-methyl and an indigenous insect growth regulator (IGR), DPE-28 (2,6-diteriarybutyl phenyl-2',4'-dinitro phenyl ether), which was reported to be effective against *Cx. quinquefasciatus* with an EI50 value of $2.2 \times 10^{-3} \text{mg/l}$ and with assessing the potential whether the nanoparticles-based formulation could improve the biological activity of the larvicides than the conventional emulsifiable concentrate formulation of the respective larvicides.

Nanoparticles are generally prepared by three methods: (i) dispersion of preformed polymers; (ii) polymerization of monomers; and (iii) ionic gelation or coacervation of hydrophilic polymers. Out of these three methods, dispersion of preformed polymers is a common technique used to prepare biodegradable nanoparticles. In this method, the polymer is dissolved in an organic solvent which is also used for dissolving the hydrophobic technical grade material which may be a drug or insecticide. The mixture of polymer and insecticide solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form an oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration.

The nanoparticles of the larvicides, temephos, pirimiphos-methyl and the IGR, DPE-28 were obtained by the method of dispersion of preformed polymers. An aqueous solution (B) of sodium lauryl sulphate (2 g) in distilled water (20 ml) was added drop-wise with magnetic stirring to the organic phase (A) obtained by mixing the solution of polyvinylpyrrolidone (PVP) (2 g) in dichloromethane (20 ml) with the solution of technical grade larvicide (0.5 g) in dichloromethane (20 ml) to yield a stable emulsion. The emulsion was stirred for 6 h and stored at $-40^\circ$C for overnight. The solvents from the freeze-dried emulsion were stripped off using a Lyophilizer at 0.1 Torr. The nanoparticles without a larvicide and with the larvicide (10%) were obtained as amorphous powder.

The properties of nanoparticles vary significantly with their size and shape, and therefore, accurate measurement of these two characteristics of nanoparticles is critical to their application. The nanoparticles obtained for the larvicides, temephos and pirimiphos-methyl and the IGR, DPE-28 were characterized using a scanning electron microscope (SEM - Hitachi S 3400) that scanned the surface of the sample in a raster pattern, utilizing an electron beam.
The sample, generally the powder, for scanning electron microscope (SEM), was dispersed in de-ionized water and a small drop of suspension was placed on a flat surface and dried using a blower. The samples of nanoparticles with or without the larvicides were dispersed in water at a concentration of <0.1% for particle size distribution analysis by Malvern Zetasizer 6.2 equipment.

The conventional 10% emulsifiable concentrate (EC) formulation of the two larvicides and the IGR, DPE-28 was prepared using technical grade insecticide as the active ingredient, xylene as the solvent and Tween-80 as the surfactant following the standard method. Laboratory bioassays for larvicidal activity of the test formulations were conducted following the WHO standard protocol. The nanoparticles without the larvicide were screened at the concentrations between 1 and 10 mg/l with respect to PVP as the active ingredient. For nanoparticles with the larvicides, five concentrations between the minimum and maximum dosages (mg/l) were chosen to get at least two concentrations giving percentage mortality <50% and two concentrations giving percentage mortality >50%. A total of 25 early instar larvae of *Cx. quinquefasciatus* (obtained from the cyclic colony maintained at VCRC) were transferred to each of the 150 ml disposable wax-coated paper cups. Each experiment was performed in four replicates with a final total of 100 larvae for each concentration. An equal number of controls were set up with 1 ml of absolute ethanol mixed with 99 ml of distilled water. Symptoms of treated larvae were observed and recorded immediately at timed intervals and larval food (yeast powder + dog biscuits) was offered to the larvae for long exposure. Mortality was recorded after 24 h of the larvicidal application. The moribund and dead larvae in four replicates were combined and expressed as percentage of larval mortality for each concentration. Dead larvae were identified when they failed to move after probing a needle. Moribund larvae were those, incapable of rising to the surface (within a reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. The test containers were held at 28 ± 2°C, 80–90% relative humidity and a photoperiod of 12 h light followed by 12 h dark (12L : 12D). In experiments, where the control mortality was between 5 and 20%, the observed test mortality was corrected using Abbott’s formula; if the control mortality exceeded 20%, the test was discarded and repeated.

The procedure reported earlier was followed for studying the IGR activity of the nano and EC formulations of DPE-28 against *Cx. quinquefasciatus* (obtained from the cyclic colony maintained at VCRC). Results of each concentration obtained from each test were subjected to computer log-probit analysis; EI₅₀ and EI₉₀ values (in ppm or mg/l) were estimated by linear regression analysis. Overall activity of the formulation was assessed as percentage inhibition of emergence (% EI) considering the mortality of all the stages based on the starting population.

The quantity of the technical grade larvicidal material was adjusted so as to obtain the nano formulation as 10% powder. The results of the SEM analysis showed that the size of the nanoparticles without the larvicides (Fig. 1) ranged from 64 to 88 nm and the size of the nanoparticles with temephos and DPE-28 ranged from 209 to 426 nm, and 48 to 88 nm, respectively (Figs. 2 & 3). Earlier, following the solvent emulsion-evaporation technique, reproducible hydrogel nanoparticles of size around 100 nm were prepared using a combination of hydroxyl propyl methyl cellulose and PVP.

The results of the particle size analysis (PSA) showed...
that the size distribution by intensity for nanoparticles without any larvicide was 186.1 nm, whereas the corresponding value for nanoparticles with pirimiphos-methyl was 120.9 nm (Figs. 4 & 5). In the case of nanoparticles with temephos, there were two peaks with the size distribution by intensity ranging between 129.2 (44.2%) and 517.4 nm (55.8%) (Fig. 6). This was similar to the observation made earlier with the stable hydroxyapatite (HAP) nanoparticles synthesized from aqueous solution of both Ca(H₂PO₄)₂ and saturated Ca(OH)₂ by precipitation method. The excellent HAP nanoparticle system could be obtained with the number-averaged particle size of 22.2 nm in 84.5% area and 54.6 nm in 15.5% area between 18.1 and 117.4 nm¹³. Thus, the preparation of nanoparticles for the larvicides and the IGR (DPE-28) was established using both SEM data and the particle distribution analysis.

The nanoparticles without the larvicide did not produce any mortality in Cx. quinquefasciatus up to 10 mg/l. The nanoparticles-based and the EC formulation of temephos, pirimiphos-methyl and DPE-28 were evaluated for larvicidal or EI activity against Cx. quinquefasciatus and the results are given in Table 1. The minimum and the maximum concentrations of the larvicides and the IGR tested to determine the LC₅₀ or EI₅₀ are also given in Table 1. The LC₅₀/El₅₀ of the nano formulation of temephos, pirimiphos-methyl and DPE-28 were 87, 73 and 771 times more than that of the EC formulation of the respective larvicides. Probably, the bind-

<table>
<thead>
<tr>
<th>Larvicide/IGR</th>
<th>Nano formulation (10%)</th>
<th>Emulsifiable concentrate (10%)</th>
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<tbody>
<tr>
<td></td>
<td>Minimum dose (mg/l)</td>
<td>Maximum dose (mg/l)</td>
</tr>
<tr>
<td>Temephos</td>
<td>0.003</td>
<td>0.04</td>
</tr>
<tr>
<td>Pirimiphos-methyl</td>
<td>0.04</td>
<td>0.14</td>
</tr>
<tr>
<td>DPE-28 {EI₅₀ (mg/l)}</td>
<td>0.01</td>
<td>0.05</td>
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ing efficiency of larvicidal particles would get enhanced with particle size from 100 nm to 10 micron as was reported earlier in relation to the drug delivery\(^\text{14}\). The nanoparticles were reported to play a definite role in taking the active molecules to the desired target especially in the drug delivery in a closed system, whereas the size of the nanoparticles has a limitation to influence positively on the biological activity of the larvicides especially in an open environment. The increased degradation of nanoparticles due to their higher mobility compared to micron-size particles resulting from the emulsifiable concentrate\(^\text{14}\) may reduce the availability of the active ingredient to produce the mortality. Thus, it could be inferred that the use of nano formulation for larvicides may have limited application in the mosquito control.

The conditions for the preparation of nanoparticles were optimized by the method of dispersion of preformed polymer using technical grade larvicide as the active ingredient, PVP as the polymer, dichloromethane as the solvent and sodium lauryl sulfate as the surfactant. The nanoparticles were characterized by both the SEM and particle size analyzer. The nanoparticles without any larvicide did not produce any significant larval mortality up to 10 mg/l. When the larvicidal activity of the nano formulation of the two larvicides used in public health, viz. temephos and pirimiphos-methyl and the IGR, DPE-28 was compared with the EC formulation of the respective larvicides, the nanoparticles were found to be less effective. The size of the particles did not have any positive influence on the biological activity and, therefore, the use of nano formulation may have limited application for the mosquito control.

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REFERENCES


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