COMBINATORIAL STUDIES ON THERMOSENSITIZATION OF NANOENCAPSULATED TEMEPHIRS AND CUSCUTA REFLEXA

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Abstract: Nanoencapsulated Temephos and Cuscuta reflexa were found effective against 3rd instar larvae of Anopheles stephensi and Culex quinquefasciatus. The present study was undertaken to investigate the effect of various temperature ranges 10°C, 15°C, 20°C and 35°C on the toxicity of the nanoformulation against both the larval stages. Temephos and Cuscuta reflexa combination were encapsulated by using polyethylene glycol (PEG). The temperature stress of 20°C on the larval stages was found to affect the susceptibility of both mosquitoes. At this temperature, the mortality of the mosquito larvae was found to be most effective than other temperature ranges. The LC$_{50}$ values were 0.0028, 0.0025 and 0.0019 mg/L against anopheline larvae and 0.0031, 0.0017 and 0.0015 mg/L against culicine larvae after 24, 48 and 72 hrs of exposure. Thus, the nanoencapsulated Temephos and Cuscuta reflexa combination would be an effective approach in mosquito management at this temperature and is not species specific.

Keywords: Anopheles stephensi, Culex quinquefasciatus, Cuscuta reflexa, Nanoencapsulation, Temephos

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INTRODUCTION

The medical importance of mosquitoes as vectors for the transmission of serious diseases that cause morbidity, mortality, economic loss, and social disruption such as malaria, lymphatic filariasis, chikungunya, dengue and other viral diseases (Becker et al. 2003). Application of synthetic insecticide based strategies for vector control results in lower efficacy of such insecticides and develops resistance in mosquito population (Brown 1986), hazardous to non-target organisms, environment and human health (Forget 1989). Recently, the use of botanical pesticides has been promoted as an alternative to synthetic insecticides, they are pest specific, cost effective, easy to use, readily biodegradable and eco-friendly (Shaalana et al. 2005). The plant C. reflexa has been reported to have synergistic properties when used in combination with Aspergillus flavus against anopheline and culicine larvae in nano-encapsulated (Bhan et al., 2014) and free form (Bhan et al., 2013). Their nanoencapsulated combination was found more effective than their free form with LC$_{50}$ 11.16, 7.07 and 1.83 mg/L against anopheline larvae and 13.37, 7.72 and 3.36 mg/L against culicine larvae after 24, 48 and 72 hrs of exposure.

Nanoencapsulation, a more sophisticated approach involves packaging the nano-scale active ingredient (pesticides) within a kind of tiny ‘envelope’ or ‘shell’. This method enables companies to manipulate the properties of the coating material of a capsule in order to control the release of the active ingredient to be delivered. In agriculture and pharmaceutical fields, the 'Controlled release' strategy is highly prized since it allows active ingredient to be absorbed more slowly at a specific location in the target organisms. Various world's leading agrochemical firms perform research on the development of new nano-scale formulations of pesticides. Due to wide potential applications of these nano and micro-formulations, they are being developed and patented by agribusiness and food corporations such as Monsanto, Syngenta and Kraft Pesticides. The merits of nano-formulation is that the pesticide dissolves more easily in water; more stable and the killing-capacity of the synthetic (herbicide, insecticide or fungicide) is optimized (Chinnamuthu and Boopathi, 2009).

Temperature is an important factor having a significant effect on the efficacy of insecticides used on fields under different temperature conditions (Brown (1987); Wang et al., (1999); Li and Luo (2004)). The toxicity of insecticides has been influenced by various environmental factors. Reason for these temperature-dependent differences may be due to changes in coverage (Wilkinson et al. 1999), insect behavior (Zubairi et al. 1964) or insecticide toxicity (Scott et al. 1995). Riveron et al. (2009) reported that the insecticides play a crucial role in the management of insect or vector-borne diseases but metabolic activities in insects responsible for insecticide degradation are highly temperature dependent. Insect’s body temperature changes with its surroundings. Therefore, environmental temperature can compromise disease vector control by influencing the toxicity of the insecticides. For example,
susceptibility of *Aedes albopictus* and *Culex restuans* mosquitoes to malathion were more at 30 °C than at 20 °C (Muturi et al. 2011). The relationship between temperature and insecticide toxicity in insects has been studied widely (Scott 1995). Although this phenomenon has been examined extensively in many insect species, few studies have compared the responses of insecticide-susceptible with insecticide-resistant strains at different temperatures. Scott (1987) compares the temperature-toxicity relationship between insecticide-susceptible and resistant German cockroaches, *Blattella germanica* (L.). His results revealed a positive temperature coefficient of toxicity for the pyrethroid, cypermethrin in an insecticide-susceptible (CSMA) German cockroach strain. However, an insecticide-resistant strain (VPIDLS) with a kdr-type mechanism exhibited a negative temperature coefficient of toxicity for cypermethrin. Conversely, Wadleigh et al. (1991) reported a negative temperature coefficient of toxicity toward cypermethrin in an unrelated insecticide-susceptible German cockroach strain, “Orlando.”

The variation of temperature sensitivity among insecticides needed more information to allow those factors which are responsible for making pest management decisions for the selection of the best product in the existing environmental conditions. The present study evaluates the effect of different temperature ranges viz. 10°C, 15°C, 20°C and 35°C on the toxicity of nanoencapsulated Temephos and *C. reflexa* combination against larvae of *An. stephensi* and *Cx. quinquefasciatus*.

**MATERIALS AND METHODS**

**Materials**

The stems of selected plant *C. reflexa* were collected from different localities of Agra. Temephos (50% EC) from Bayer and Polyethylene glycol 6000 (PEG) was purchased from Merck. The water used for all experiments was deionized and all other reagents used were commercially available and were of analytical grade.

**Culture of Mosquito**

The mosquito vectors, *An. stephensi* and *Cx. quinquefasciatus* were reared in the laboratory, maintained continuously at 27±2°C and 70-80% relative humidity under a photoperiod of 14:10 hrs (light/dark) without exposure to pathogens or insecticides. Freshly soaked deseeded raisins were supplied to adults and powdered brewer’s yeast to larvae. For egg maturation, periodic blood meals were provided to female mosquitoes by keeping restrained albino rats in the cages. The eggs were collected in a petri dish lined with moist Whatman filter paper and were
allowed to hatch in trays filled with de-chlorinated water. Larvae were fed with a mixture of yeast powder and ground dog biscuits. The pupae formed were collected and transferred to the cloth cages for adult emergence. Freshly molted larvae were continuously collected for the larvicidal experiments.

**Preparation of phytoextract**

The stems of *C. reflexa* were collected from the different localities of Agra. The collected stems were then washed in running tap water and allowed to dry in shade. The shade dried stems were then crushed mechanically and subjected to extraction with petroleum ether in a soxhlet apparatus for 72 hrs. Extract was concentrated by removing the solvent by vacuum rotatory evaporator. The extract obtained as a thick viscous paste was completely evaporated to dryness at room temperature and kept in refrigerator below 5 °C after weighing until further use.

**Encapsulation of Temephos and *C. reflexa* nanoparticles**

The encapsulation of nanoparticles combination was conducted by using melt-dispersion method (Peng et al., 2008). About 46.0 g of PEG (6000) was heated at 65 °C, to this melted part 4 g of Temephos and *C. reflexa* combination were added to obtain nanoparticles. The nanof ormulation was stirred gently with the glass rod to ensure even distribution of the mixture. The mixture was then cooled at room temperature, grounded completely in a mortar and sieved using a 200 mesh sieve. Finally, the nanopesticides were then placed in airtight, self-sealable polyethylene pouches and stored at 25 °C in desiccators containing calcium chloride to prevent moisture absorption prior to experiments.

**Thermosensitization of the encapsulated nanosynthetic and phyto pesticide combination**

In order to evaluate the larvicidal efficacy of the encapsulated nanopesticide combination, Twenty, 3rd instar larvae, *An. stephensi* and *Cx. quinquefasciatus* were collected separately. They were placed in a 250 mL beaker with 200 mL of water and then transferred gently to different working test concentrations with a control individually. A certain mass of NPs was placed in a 50 mL beaker containing deionized water to prepare stock solution of 1000 mg/L independently. A control (blank) sample was used with the same nanoparticle composition and larvae number, however, with no pesticide loading present. All experiments were arranged in triplicates and divided into five batches and were exposed to temperatures ranges 10°C, 15°C, 20°C and 35°C. A small aliquot of yeast powder was supplied for nutrition. Daily loss of water from experimental series was adjusted by adding required quantity of tap water up to the marking on the experimental beakers. The larval mortality in both treated and controls were monitored after 24, 48 and 72 hrs of exposure. The larvae were considered dead if they were immobile and unable to reach the water surface (Macedo et al. 1997).
Statistical data analysis

The recorded mortality data after 24, 48 and 72 hrs of exposure period for each experiment was analysis by using probit analysis (Finney 1971). Experiments with more than 20% mortality in control were discarded and if mortality ranging 5-20% in control, the mortality data were corrected by applying Abbot’s formula (Abbot 1925) so as to remove the factors responsible for larval mortality other than the nanopesticides. The lethal concentration values for 50% and 90% mortality (LC50 and LC90) with other statistical values were determined at 95% fiducial confidence intervals along with relative toxicity and chi-square.

Characterisation of nanoparticles

Transmission Electron Microscopy

The morphological study of the nano-encapsulated combination was determined by Transmission Electron Microscopy (TEM). For TEM studies, a small amount of nanoformulation was dissolved in deionised water. A drop of this solution was then placed on a copper grid and dried in vaccum. The micrographs were obtained using Philips Morgagni (M-268).

Particle size and distribution

The nanoparticle size and their size distribution were analyzed with the Nanozetasizer (Malvern). To dilute the sample deionised water was added with 0.5 g in 50 mL and filtered through a millipore filter to avoid any contamination. For the accuracy of the size, each measurement was performed in triplicate.

RESULTS

Characterization of nanoparticles

The mean size of the encapsulated nanoformulation combination was 129.5 nm and its size distribution was showed in figure (Fig. 1). The TEM analysis revealed that the nanoparticle predominates with spherical morphology. Most of the nanoparticles were irregular shaped and having smooth edges (Fig. 2).
Figure 1 The particle size distribution histogram for polyethylene glycol (PEG) loaded nanoencapsulated Temephos and *C. reflexa* combination.

Figure 2 The TEM micrograph for polyethylene glycol (PEG) loaded nanoencapsulated Temephos and *C. reflexa* combination.
Effect of thermosensitization on the efficacy of encapsulated nanopesticide combination

The thermalsensitization of the toxicity of the encapsulated nanopesticide at different temperatures (10°C, 15°C, 20°C and 35°C) was evaluated against both larvae. Table 1 represents the LC50 for 10°C was 0.0043, 0.0038 and 0.0031 mg/L and LC90 0.015, 0.015 and 0.014 mg/L after 24, 48 and 72 hrs. The LC50 for 15°C was 0.0040, 0.0039 and 0.0036 mg/L and at LC90 0.016, 0.018 and 0.017 mg/L after 24, 48 and 72 hrs. The LC50 for 20°C was 0.0028, 0.0025 and 0.0019 mg/L and at LC90 0.0069, 0.0067 and 0.0062 mg/L after 24, 48 and 72 hrs. At 35°C, LC50 was 0.0055, 0.0048 and 0.0038 mg/L and LC90 0.021, 0.017 and 0.016 mg/L after 24, 48 and 72 hrs against anopheline larvae (Fig. 3).

Table 1 Thermalsensitization of the most potent encapsulated nanopesticide at LC50 and LC90 against anopheline larvae under different temperature conditions

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Exposure Period (hrs.)</th>
<th>Chi-square</th>
<th>Regression equation</th>
<th>LC50±SE (UL-LL) (mg/L)</th>
<th>Amount of larvicide released (mg/L) at LC50</th>
<th>Relative toxicity (with respect of exposure period)</th>
<th>LC90±SE (UL-LL) (mg/L)</th>
<th>Amount of larvicide released (mg/L) at LC90</th>
<th>Relative toxicity (with respect of exposure period)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C</td>
<td>24</td>
<td>0.889</td>
<td>2.291±x+8.135</td>
<td>0.0043±0.0009 (0.0060-0.0026)</td>
<td>0.00034 1.279</td>
<td>0.0155±0.0059 (0.0272-0.0039)</td>
<td>0.0012 1.348</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.581</td>
<td>2.096±x+7.979</td>
<td>0.0038±0.0008 (0.0054-0.0022)</td>
<td>0.00030 1.263</td>
<td>0.0155±0.0062 (0.0276-0.0033)</td>
<td>0.0012 1.174</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.342</td>
<td>1.977±x+7.968</td>
<td>0.0031±0.0007 (0.0045-0.0018)</td>
<td>0.00025 1.225</td>
<td>0.0140±0.0057 (0.0252-0.0029)</td>
<td>0.0011 1.257</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15°C</td>
<td>24</td>
<td>4.032</td>
<td>2.153±x+8.0075</td>
<td>0.0040±0.0008 (0.0055-0.0025)</td>
<td>0.00032 1.375</td>
<td>0.0158±0.0061 (0.0277-0.0039)</td>
<td>0.0013 1.323</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>5.709</td>
<td>1.944±x+7.719</td>
<td>0.0039±0.0008 (0.0056-0.0023)</td>
<td>0.00031 1.231</td>
<td>0.0182±0.0082 (0.0342-0.0022)</td>
<td>0.0014 1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>4.261</td>
<td>1.865±x+7.686</td>
<td>0.0036±0.0008 (0.0052-0.0021)</td>
<td>0.00029 1.055</td>
<td>0.0176±0.0081 (0.0335-0.0018)</td>
<td>0.0014 1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20°C</td>
<td>24</td>
<td>4.163</td>
<td>3.278±x+10.089</td>
<td>0.0028±0.0004 (0.0036-0.0020)</td>
<td>0.00022 1.964</td>
<td>0.0069±0.0018 (0.0104-0.0033)</td>
<td>0.00055 3.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>3.734</td>
<td>3.024±x+9.836</td>
<td>0.0025±0.0004 (0.0033-0.0017)</td>
<td>0.0002 1.92</td>
<td>0.0067±0.0018 (0.0102-0.0030)</td>
<td>0.00054 2.716</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>3.395</td>
<td>2.573±x+9.386</td>
<td>0.0019±0.0004 (0.0027-0.0012)</td>
<td>0.00015 2</td>
<td>0.0062±0.0019 (0.0099-0.0025)</td>
<td>0.00049 2.839</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>SE (Days)</td>
<td>SE (Temp)</td>
<td>SE (Conc)</td>
<td>SE (Time)</td>
<td>SE (Temp)</td>
<td>SE (Conc)</td>
<td>SE (Time)</td>
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</tr>
<tr>
<td>35°C</td>
<td>24</td>
<td>0.134</td>
<td>2.224x+7.794</td>
<td>0.0055±0.0012</td>
<td>(0.0079-0.0032)</td>
<td>0.00044</td>
<td>1.00</td>
<td>0.0209±0.0105</td>
<td>(0.0414-0.0003)</td>
</tr>
<tr>
<td>48</td>
<td>0.054</td>
<td>2.369x+8.125</td>
<td>0.0048±0.0009</td>
<td>(0.0066-0.0029)</td>
<td>0.00038</td>
<td>1.00</td>
<td>0.0167±0.0073</td>
<td>(0.0310-0.0023)</td>
<td>0.0013</td>
</tr>
<tr>
<td>72</td>
<td>0.068</td>
<td>2.099x+7.969</td>
<td>0.0038±0.0008</td>
<td>(0.0054-0.0022)</td>
<td>0.00030</td>
<td>1.00</td>
<td>0.0157±0.0072</td>
<td>(0.0298-0.0016)</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

SE: Standard Error; UL: Upper Fiducial Limit; LL: Lower Fiducial Limit

![Graph showing lethal concentrations at different temperatures and time points]
Figure 3 a) and b) showing thermalsensitization of the nanoencapsulated combination at different temperature conditions at LC$_{50}$ and LC$_{90}$ against anopheline larvae.

Table 2 represents the LC$_{50}$ for 10°C was 0.439, 0.262 and 0.186 mg/L and LC$_{90}$ 2.51, 1.35 and 0.95 mg/L after 24, 48 and 72 hrs. For 15°C, LC$_{50}$ was 0.24, 0.18 and 0.15 mg/L and at LC$_{90}$ 0.97, 0.62 and 0.44 mg/L after 24, 48 and 72 hrs. The LC$_{50}$ at 20°C was 0.0031, 0.0017 and 0.0015 mg/L and at LC$_{90}$ 0.0073, 0.0040 and 0.0036 mg/L after 24, 48 and 72 hrs. The amount of nanopesticide released for 35°C at LC$_{50}$ was 0.0096, 0.0055 and 0.0036 mg/L and LC$_{90}$ 0.058, 0.031 and 0.016 mg/L after 24, 48 and 72 hrs against culicine larvae (Fig.4).

Table 2 Thermalsensitization of the most potent encapsulated nanopesticide at LC$_{50}$ and LC$_{90}$ against culicine larvae under different temperature conditions

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Exposure Period (hrs.)</th>
<th>Chi-square</th>
<th>Regression equation</th>
<th>LC$_{50}$±SE (UL-LL) (mg/L)</th>
<th>Amount of larvicide released (mg/L) at LC$_{50}$</th>
<th>Relative toxicity (with respect of exposure period)</th>
<th>LC$_{90}$±SE (UL-LL) (mg/L)</th>
<th>Amount of larvicide released (mg/L) at LC$_{90}$</th>
<th>Relative toxicity (with respect of exposure period)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C</td>
<td>24</td>
<td>2.307</td>
<td>1.691x+3.914</td>
<td>0.439±0.118 (0.671-0.206)</td>
<td>0.035</td>
<td>1.00</td>
<td>2.513±1.255 (4.973-0.0523)</td>
<td>0.201</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>1.603</td>
<td>1.795x+4.249</td>
<td>0.262±0.066 (0.390-0.133)</td>
<td>0.021</td>
<td>1.00</td>
<td>1.355±0.602 (2.534-0.1753)</td>
<td>0.108</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>2.174</td>
<td>1.814x+4.509</td>
<td>0.186±0.047 (0.279-0.094)</td>
<td>0.015</td>
<td>1.00</td>
<td>0.948±0.399 (1.730-0.165)</td>
<td>0.076</td>
<td>1.00</td>
</tr>
<tr>
<td>15°C</td>
<td>24</td>
<td>1.010</td>
<td>2.135x+4.176</td>
<td>0.243±0.054 (0.349-0.137)</td>
<td>0.019</td>
<td>1.804</td>
<td>0.969±0.359 (1.673-0.265)</td>
<td>0.077</td>
<td>2.594</td>
</tr>
</tbody>
</table>
### Table 1: Lethal Concentrations (LC50) for \( T = 20^\circ C, 35^\circ C \)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time (h)</th>
<th>LC50 (mg/L)</th>
<th>SE (mg/L)</th>
<th>UL (mg/L)</th>
<th>LL (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T = 20^\circ C )</td>
<td>24 hrs</td>
<td>5.003</td>
<td>0.0031 ± 0.0004</td>
<td>0.00025</td>
<td>141.484</td>
</tr>
<tr>
<td></td>
<td>48 hrs</td>
<td>2.287</td>
<td>0.0017 ± 0.0003</td>
<td>0.00014</td>
<td>154.059</td>
</tr>
<tr>
<td></td>
<td>72 hrs</td>
<td>1.857</td>
<td>0.0015 ± 0.0002</td>
<td>0.00012</td>
<td>121.4</td>
</tr>
<tr>
<td>( T = 35^\circ C )</td>
<td>24 hrs</td>
<td>1.621</td>
<td>0.0096 ± 0.0026</td>
<td>0.00077</td>
<td>45.687</td>
</tr>
<tr>
<td></td>
<td>48 hrs</td>
<td>1.761</td>
<td>0.0055 ± 0.0014</td>
<td>0.00044</td>
<td>47.618</td>
</tr>
<tr>
<td></td>
<td>72 hrs</td>
<td>0.866</td>
<td>0.0036 ± 0.0009</td>
<td>0.00029</td>
<td>51.75</td>
</tr>
</tbody>
</table>

SE: Standard Error; UL: Upper Fiducial Limit; LL: Lower Fiducial Limit

### Graph

- **24 hrs**
- **48 hrs**
- **72 hrs**

Graph showing lethal concentrations at different temperatures and time intervals.
b)

Figure 4 a) and b) showing thermal sensitization of the nanoencapsulated combination at different temperature conditions at LC$_{50}$ and LC$_{90}$ against culicine larvae.

**DISCUSSION**

Present investigation indicates that the nanoencapsulated combination was influenced differently by temperature as it was one of the most extrinsic factors affecting toxicity of the pesticides. Toxicity of nanoencapsulated mixture was influenced by temperature more effectively at 20°C at LC$_{50}$ was 0.0028, 0.0025 and 0.0019 mg/L against anopheline and was 0.0031, 0.0017 and 0.0015 mg/L against culicine larvae after 24, 48 and 72 hrs of treatment. However, at 35°C the toxicity of nanoencapsulated combination was the lowest and it almost loses its efficiency in case of anopheline larvae but in case of culicine larvae at 10°C the toxicity is lowest. It is interesting to note that the value of LC$_{50}$ and LC$_{90}$ values varied differently between the tested temperatures. Some of them rose or fell gradually and some fluctuated with temperature change. Similar work was found by Punzo (1993) who studied the effect of different temperatures on cis-cypermethrin to *Spodoptera frugiperda* (Lepidoptera: Noctuidae). His result revealed that the lethal action of cis-cypermethrin decreases with the rise of temperature ranges 15–27°C, but at 27–38°C, it showed to be a positive temperature coefficient insecticide. Further, the thermosensitisation of the combination evaluated may be either by increasing the interactivity of the combatants affecting the chemical nature of the pesticide or by influencing the physiological environment of the target organisms by bringing the changes in their hormonal status, biotransformation or translocation of the chemicals. The toxicity of the combination is thermo dependent, which is in consonance with the work of Watters et al. (1983), Fisher and Wadleigh (1985), Thaung and Collins (1986), Scott (1987), Subramanyam and Cutkomp (1987) and Wadleign et al. (1991).
Various other workers have studied different thermal responses existing between insecticides and insect species. The thermosensitisation of three pyrethroid insecticides cypermethrin, fenvalerate and permethrin in the management of Tribolium castaneum was reported by Watters et al. (1983). Fisher and Wadleigh (1985) studied the thermal effect on the acute toxicity and uptake of lindane by Chironomus riparius. Joint effects of temperature and insecticides on mortality and fecundity of Sitophilus oryzae was observed by Thaung and Collins (1986). Scott (1987) observed the thermosensitisation of two pyrethroids bioallethrin and cypermethrin against susceptible and kdr-resistant strains of Blatella germanica. The influence of post treatment temperature on the toxicity of pyrethroids; bioallethrin, cypermethrin, cyfluthrin, d-phenothenir, fenvalerate and flucythrinate against Cadra cautella, Plodia interpunctella, Prostephanus truncates, Rhyzopertha dominica and Tribolium confusum was described by Subramanyam and cutkomp (1987). Yadwad and Kallapur (1988) has studied the effect of temperature on fenitrothion treatment with reference to Achaea janata, Bombyx mori and Mythimna separate. Thermosensitisation of 10 pyrethroids including cyfluthrin, λ-cyhalothrin, cypermethrin, d-phenothenir, fenvalerate, fenvalerate, fluvalinate, permethrin, resmethrin and tralomethrin against B. germanica was studied by Wadleigh et al. (1991). Garbalunski (1994) investigated the influence of temperature on the activity of chlorinated hydrocarbons, organophosphate, carbonate compounds, pyrethroids and biological insecticide, Bacillus thuringiensis. Malinowski and Garbalinski (1995) studied the influence of temperature on the activity of pyrethroids (deltamethrin and alphamethrin), carbonates (Carbosulfam) and organophosphates (chlorpyrifos) against Hyllobius abietis. Soma et al. (1995) has evaluated the response of stored grain insects with reference to S. zeamai, S. granaries and T. confusum to carbon dioxide toxicity and its dependence on temperature. The thermal effect along with concentration, light etc. on infection of mosquito larvae by Lagenidium giganteum was observed by Suh and Axtell (1999). Sharma (2002) investigated the thermosensitization larvicidal activity of Artimisia annua and Azadirachta indica to anopheline and culicine larvae.

Musser and Shelton (2005) showed that two pyrethroids (λ-cyhalothrin and bifenthrin) and spinosad had negative temperature coefficients, while methomyl had no temperature coefficient against Ostrinia nubilalis (Lepidoptera: Crambidae). Sririgiraju et al (2010) evaluated acephate, methomyl, and imidaclorpid as exhibiting positive temperature coefficients, with the exception of λ-cyhalothrin against tobacco aphid (Aphidae: Hemiptera). Ma et al. (2012) has evaluated the toxicity of eight conventional insecticides to the third-instar Apolygus lucorum was measured at 15°C, 20°C, 25°C, 30°C, and 35°C. Khan and Akram (2014) determined the effect of temperature range, 20–34°C on the toxicity of seven insecticides from organophosphate (chlorpyrifos, profenofos), pyrethroid (cypermethrin, deltamethrin) and new insecticides, emamectin benzoate, fipronil, spinosad against Musca domestica.
Temperature influences the effectiveness of insecticides in a non-constant way. Various workers have found that an increase in temperatures enhances efficacy of organophosphates, spinosad, or abamectin but this is not always true with pyrethroids (Athanassiou et al. (2008); Kavallieratos et al. (2009)). Higher temperatures also reduce residual life and deposition of insecticides (Bobé et al. (1998); Arthur et al. (1992)). More the temperature differences in regions, the harder choice will be made in insect management. Mosquitoes and pests were distributed across a wide range where the temperature varied a lot. When insecticides from different classes are available to control a pest, knowledge of a product’s temperature coefficient will be required for pest managers to select a product that is efficacious under given environmental conditions (Ma et al., 2012).

In conclusion, toxicity of nanoencapsulated combination to mosquito larvae revealed its influence at different temperature range tested. The results could be helpful in designing effective nano-based management plans for mosquito larvae control in summer and winter seasons at favorable temperature conditions.

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