RELATIVE LARVICIDAL POTENTIALITY OF CERTAIN ENCAPSULATED SYNTHETIC, PLANT AND FUNGAL PESTICIDES AGAINST MOSQUITO LARVAE

ABSTRACT

Mosquitoes are one of the serious scourges inflicted upon humanity. They pose the major public health menace because of their ability to transmit various pathogens causing dreadful diseases such as malaria, filariasis, dengue, chikungunya and Japanese encephalitis which afflict millions of people worldwide. In addition, mosquito-bites can cause severe skin irritation through an allergic reaction to the mosquito's saliva causing the eruptions and itching. Thus, mosquito is the most nuisances creating and irritating dipeteran. WHO, therefore, designated them as enemy number one to mankind (WHO, 1996).

In recent decade, Nanotechnology becomes a promising field of research which opens up a wide array of opportunities. Nanotechnology is the production, manipulation and the use of materials ranging in size from less than a micron to that of individual atoms. Nanoparticles are considered as the building blocks of nanotechnology. The synthesis of nanoparticles especially metallic (silver, gold, zinc, alumina, titanium, platinum and silica/silicon) and polymeric nanoparticles has accrued utmost interest over the past decade owing to their unique properties that make them applicable in different fields of science and technology.

Pesticides in nanoparticles form is known as nanopesticides. We get nanopesticides either pesticide loaded with metallic nanoparticles or by encapsulation method. The pesticidal particles, slowly released from encapsulated form are nanopesticides. The nanopesticides which are of plant origin are known as nanophytopesticides.

Encapsulation formulations have been revolutionized the application of pesticides due to the development of nanotechnology in insect pest management. Nanoencapsulation is a process through which a chemical such as insecticide is slowly and efficiently released for insect pest control. The nanoencapsulated pesticides show meritorious properties in the
field of insect pest management. They require lesser pesticide quantity which reduces the application dosages resulting in reduced input costs, environmental pollution and causing less impact on non-target organisms. Moreover, they are for safer storage due to reduced flammability, helps in occlusion of pesticide odor, released in a controlled and targeted fashion. The liquid pesticides rendering into powders and prevent clumping which improves mixing and more reactive due to their increased surface area. Further, they protect active ingredient from degradation by direct exposure to severe environmental conditions such as light, temperature etc., thereby increasing its shelf life.

The bioefficacy of encapsulated and non-encapsulated synthetic pesticides (Temephos and Imidaclorpid), plants (Solanum xanthocarpum and Cuscuta reflexa) and Fungi (Aspergillus flavus and Fusarium sporotrichioides) is determined and their different combinations are also tested to determine the most potent combination against mosquito larvae (Anopheles stephensi and Culex quinquefasciatus). The effect of light and temperature on the toxicity of the most potent encapsulated nanopesticide was evaluated to determine the favorable conditions for effective application against the target organisms. Further, the impact of encapsulated nanopesticide on aquatic non-targets (Daphnia magna and Cypris spp.) was also observed to examine the eco-friendly nature of the most potent encapsulated nanopesticide studied and further to enhance the importance of present investigation in mosquito management.

The selected plants, S. xanthocarpum (roots) and C. reflexa (stems) were collected from the availability area of Agra. The roots and the stems were separated from the plant and washed in running tap water and were dried in the shade. Dried roots were chopped in small pieces of about 1 cm size using a Falcon-Stem Cutter. The roots and stems were subjected to extraction with petroleum ether, hexane and methanol subsequently in a soxhlet apparatus for 72 hours. Extracts were separated from their respective solvents by vaccum rotatory evaporator to get pure residues. The extracts were finally weighed and kept in refrigerator below 5°C until further use.

**Selected fungi were cultured** by obtaining the strains of A. flavus from Microbial Type Culture Collection and Gene Bank, Chandigarh, India (MTCC No.- 1973) and
F. sporotrichioides (MTCC No.- 1894) and stored at 4°C. Prior to testing on mosquito larvae they were cultured on PDA- peptones (20g/l), dextrose (40g/l), agar (20g/l) plate’s separately and incubated in BOD (Biochemical Oxygen Demand) at 28°C for 7 days. After seven days both were visually characterized. A. flavus and F. sporotrichioides isolates were then subcultured on Czepak solution agar media (sucrose-30g/l, agar-15g/l, NaNO3-2g/l, K2HPO4-1g/l, KCl-0.5g/l, MgSO4.7H2O-0.5g/l, FeSO4.7H2O-0.01g/l at pH-7.3± 0.2) to obtain pure cultures. Species were determined using microscopic features (Klich and Pitt, 1994). All fungal isolates were kept at 4°C for further analysis. Further for the extraction of mycotoxins, isolates of A. flavus and F. sporotrichioides were cultured in a 250 ml flask containing 100 ml of sterile YES (Yeast Extract Sucrose) liquid medium (20% sucrose and 5% yeast extract) and incubated separately for 7-10 days in the dark at 27-30°C without agitation according to Mallek et al., (1993). The residue were finally weighed and kept in refrigerator at 4°C until further use.

The selected synthetic pesticides, Temephos were taken from the District Malaria Office, Agra and Imidacloprid were purchased from the local market. For conducting their bioassay their stock solutions of desired concentrations were prepared by diluting the pesticides independently in dechlorinated tap water. Different test concentrations were prepared for the exposure of mosquito larvae by further diluting these stocks. One milliliter of these test concentrations was added into 249 ml of water in 500 ml capacity of glass beakers to obtained different working test concentration for the exposure of mosquito larvae. After 24 hrs of acclimatization in lab condition twenty, 3rd instar mosquito larvae, An. stephensi and Cx. quinquefasciatus were collected and exposed to each working concentration independently. Each experiment was arranged in triplicates to minimize error with a control at an ambient temperature 27±2°C and 70–80% relative humidity. The larvae were supplied with small aliquot of powdered brewer’s yeast for nutrition. Loss of water was adjusted by adding required quantity of tap water upto the marked level of test. All the experiments were conducted according to WHO standard procedure (2005).
The synthesis of nanopesticides formulations were done by using melt-dispersion method. PEG 6000, (Polyethylene Glycol) was heated in different four parts (49.5, 49.0, 48.0 and 46.0 g) separately at 65°C. After melting, 1%, 2%, 4%, and 8% of pesticide were mixed separately with the different parts of melted PEG and stirred gently with the glass rod to ensure even distribution of the mixture. The mixture was cooled at room temperature and the mixture was grounded completely in a mortar and sieved using a 200 mesh sieve. The powders are placed in airtight, self-sealable polyethylene pouches and stored at 25°C in desiccators containing calcium chloride to prevent moisture absorption prior to further experiments.

The bioefficacy of encapsulated synthetic pesticides (Temephos and Imidacloprid), were done by dissolving them in deionised water (w/v) to make stock solutions of nanopesticides independently. Bioassay of encapsulated synthetic pesticides was depicted as mentioned earlier.

The bioassay of plant extracts, the pure residues were dissolved in ethanol to get stock solutions. Different desired test concentrations were prepared by diluting these stocks in ethanol. Methodology of bioassay of plant extracts, their encapsulation and bioassay of encapsulated plant extracts were same as conducted for the synthetic pesticides.

For the bioassay of fungi, the fungal residues were dissolved in ethanol independently to prepare stock solutions and different test concentrations were made by further dilution of these stocks. Methodology of bioassay of fungal extracts, their encapsulation and bioassay of encapsulated fungal extracts were same as conducted for the synthetic pesticides.

The most potent combinations among synthetic, phyto, microbial pesticides were established and their combinatorial studies were assessed individually. For the bioassay of non-encapsulated pesticides synthetic pesticide were kept as standard, the stock was mixed with the stock of phyto or fungal extract in ratios of 1:1, 1:2 and 1:4 individually.
Test concentrations for each mixed formulated ratio were prepared by further diluting the combination mixture in water.

The procedure for the synthesis of encapsulated combinations was same as that for the encapsulated individuals. The bioassays of all the encapsulated combinations were conducted according to the previous methodology.

All the experiments were devised according to WHO standard procedure (2005). Mortality observations were recorded after 24, 48 and 72 hrs of exposure for each experiment. The dead and moribund larvae were recorded as larval mortality. The mortality of larvae was determined by observing the movement of the larvae after the treatment period. The mortality was also recorded in the control. The experiment was discarded if the larval mortality exceeded 20 % in control and repeated again so as to keep the mortality in control below 20 %. Recorded mortality data were then subjected for calculation of lethal concentration 50 (LC\(_{50}\)) and 90 (LC\(_{90}\)) according to probit analysis. Prior to LC\(_{50}\) & LC\(_{90}\) calculation, Abbott's formula was applied if the percent mortality in control ranged between 5 - 20% to percent mortality. If the percent larval mortality was below 5%, then these were treated as non-significant and thus no correction was required.

The permanent slides of untreated and treated mosquito larvae were prepared by standard procedure for study of morphological changes in the treated mosquito larvae.

The larvicidal efficacy of Temephos and Imidacloprid against An. stephensi and Cx. quinquefasciatus reveals that Temephos were more effective with the LC\(_{50}\) values for Temephos were 0.0025, 0.0023 and 0.0020 mL/L and the LC\(_{90}\) values were 0.0052, 0.0040 and 0.0036 mL/L after 24, 48 and 72 hrs against anopheline larvae. The LC\(_{50}\) values for temephos were 0.0060, 0.0055 and 0.0042 mL/L and the LC\(_{90}\) values were 0.018, 0.016 and 0.011 mL/L after 24, 48 and 72 hrs of treatment against culicine larvae.

The larval mortality after the treatment of different encapsulated nano-formulations of temephos and imidacloprid reveals that PEG with optimum loading of 8% temephos were most potent among encapsulated synthetic pesticides. The amount of nanopesticide releases for 8% formulation, at LC\(_{50}\) was 0.013, 0.009 and 0.001 mg/L and
at LC$_{90}$ it was 0.067, 0.028 and 0.012 mg/L 24, 48 and 72 hrs, against anopheline larvae and in case of culicine larvae The amount of nanopesticide releases for 8% formulation, at LC$_{50}$ was 0.013, 0.010 and 0.003 mg/L and at LC$_{90}$ it was 0.043, 0.047 and 0.017 mg/L 24, 48 and 72 hrs, accordingly.

The larvicidal potentiality of different extracts of _C. reflexa_ and _S. xanthocarpum_ against _An. stephensi_ and _Cx. quinquefasciatus_ reveals that the PEE (petroleum ether extract) of _C. reflexa_ were most effective with the LC$_{50}$ values 39.251, 33.180 and 20.032 mg/L and the LC$_{90}$ values were 292.771, 229.935 and 134.976 mg/L after 24, 48 and 72 hrs against anopheline larvae. The LC$_{50}$ values were 48.625, 31.869 and 21.667 mg/L and the LC$_{90}$ values were 266.272, 175.041 and 156.014 mg/L after 24, 48 and 72 hrs of treatment against culicine larvae.

The larval mortality after the treatment of their different encapsulated nano-formulations reveals that PEG with optimum loading of 1% PEE of _C. reflexa_ were most potent among other encapsulated nano-phytosticides. The amount of nano-phytosticide releases for 1% formulation, at LC$_{50}$ was 14.479, 11.560 and 8.501 mg/L and at LC$_{90}$ it was 84.285, 88.254 and 75.256 mg/L 24, 48 and 72 hrs, against anopheline larvae and in case of culicine larvae the amount of nano-phytosticide releases for 1% formulation, at LC$_{50}$ was 2.451, 1.765 and 1.446 mg/L and at LC$_{90}$ it was 54.029, 25.643 and 21.103 mg/L 24, 48 and 72 hrs, respectively.

The larvicidal activity of mycotoxins of _A. flavus_ and _F. sporotrichoides_ against _An. stephensi_ and _Cx. quinquefasciatus_ illustrates that the _A. flavus_ were most effective with the LC$_{50}$ values 10.872, 8.153 and 7.049 mg/L and the LC$_{90}$ values were 33.233, 27.286 and 19.550 mg/L after 24, 48 and 72 hrs against anopheline larvae. The LC$_{50}$ values were 13.616, 14.347 and 10.027 mg/L and the LC$_{90}$ values were 70.313, 67.474 and 45.691 mg/L after 24, 48 and 72 hrs of treatment against culicine larvae.

The larval mortality after the treatment of their different encapsulated nano-formulations reveals that PEG with optimum loading of 8% _A. flavus_ were most potent among other encapsulated nano-fungalpesticides. The amount of nano-fungalpesticide
releases for 8% formulation, at LC₅₀ was 7.814, 7.286 and 6.213 mg/L and at LC₉₀ it was 40.525, 39.733 and 36.233 mg/L 24, 48 and 72 hrs, against anopheline larvae and in case of culicine larvae the amount of nano-fungal pesticide releases for 8% formulation, at LC₅₀ was 12.236, 10.820 and 8.296 mg/L and at LC₉₀ it was 75.992, 69.254 and 57.477 mg/L 24, 48 and 72 hrs, respectively.

The bioefficacy of different combinations, bipartite and tripartite of most potent synthetic, phyto and microbial pesticides reveals that the bipartite ratio (1:1) of Temephos and crude petroleum ether extract of C. reflexa were most effective among all the combinations tested against both the mosquito larvae. The combinatorial ratio 1:1 has the LC₅₀ value 0.0013, 0.0010 and 0.0009 mg/L after 24, 48 and 72 hrs. The LC₉₀ values were 0.0103, 0.0067 and 0.0042 mg/L after 24, 48 and 72 hrs of exposure, accordingly against anopheline larvae while LC₅₀ value 0.0016, 0.0014 and 0.0013 mg/L after 24, 48 and 72 hrs. The LC₉₀ values were 0.0082, 0.0085 and 0.006 mg/L after 24, 48 and 72 hrs of exposure, against culicine larvae.

The encapsulated combinatorial studies have demonstrated that the larvicidal potentiality of PEG with optimum loading of 8% combination of temephos and C. reflexa extract have LC₅₀ values 0.0043, 0.0015 and 0.0006 mg/L and LC₉₀ values 0.0231, 0.0061 and 0.0029 mg/L after 24, 48 and 72 hrs against anopheline larvae. While in case of culicine larvae the LC₅₀ values 0.030, 0.0032 and 0.0011 mg/L and LC₉₀ values 0.3523, 0.0186 and 0.0074 mg/L after 24, 48 and 72 hrs were most effective than other nano formulations.

The morphological studies reveal that under control there is no change in the morphology of the larvae, anopheline and culicine. In treated anopheline and culicine larvae antennae and bristles are disturbed. Alimentary canal and heamolymphatic tissues were partly damaged. Body segments were loosen at their joints.

The effects of different parameters (Light and Temperature) were observed on the bioefficacy of the most potent encapsulated nanopesticide. The photo period conditions were observed under fluorescent (Visible range), dark and UV-radiations (1
hour, 2 hours and 4 hours) against both larvae. The fluorescent (visible range) light affected with LC$_{50}$ 0.0019, 0.0011 and 0.0009 mg/L and LC$_{90}$ 0.0086, 0.0025 and 0.0017 mg/L after 24, 48 and 72 hrs. The darkness affected with LC$_{50}$ 0.0077, 0.0055 and 0.0048 mg/L and LC$_{90}$ 0.0658, 0.0429 and 0.0349 mg/L after 24, 48 and 72 hrs. The UV-1 treated concentration affected with with LC$_{50}$ 0.0029, 0.0014 and 0.0012 mg/L and LC$_{90}$ 0.0185, 0.0105 and 0.0054 mg/L after 24, 48 and 72 hrs. The UV-2 treated concentration affected with LC$_{50}$ 0.0035, 0.0013 and 0.0013 mg/L and LC$_{90}$ 0.0139, 0.0042 and 0.0025 mg/L after 24, 48 and 72 hrs. The UV-4 treated concentration affected with LC$_{50}$ 0.0045, 0.0037 and 0.0026 mg/L and LC$_{90}$ 0.0150, 0.0149 and 0.0099 mg/L after 24, 48 and 72 hrs against anopheline larvae. The fluorescent (visible range) light affected with LC$_{50}$ 0.0058, 0.0045 and 0.0028 mg/L and LC$_{90}$ 0.0471, 0.0404 and 0.0130 mg/L after 24, 48 and 72 hrs. The darkness affected with LC$_{50}$ 0.0742, 0.0625 and 0.0504 mg/L and LC$_{90}$ 0.4730, 0.3901 and 0.2826 mg/L after 24, 48 and 72 hrs. The UV-1 treated concentration affected with LC$_{50}$ 0.0044, 0.0031 and 0.0025 mg/L and LC$_{90}$ 0.0213, 0.0188 and 0.0156 mg/L after 24, 48 and 72 hrs. The UV-2 treated concentration affected with LC$_{50}$ 0.0052, 0.0040 and 0.0032 mg/L and LC$_{90}$ 0.0198, 0.0182 and 0.0089 mg/L after 24, 48 and 72 hrs. The UV-4 treated concentration affected with LC$_{50}$ 0.0054, 0.0049 and 0.0031 mg/L and LC$_{90}$ 0.0135, 0.0140 and 0.0103 mg/L after 24, 48 and 72 hrs against culicine larvae.

The effect of different temperatures (10°C, 15°C, 20°C and 35°C) on the bioefficacy of the most potent encapsulated nanopesticide was evaluated against both larvae. The LC$_{50}$ for 10°C was 0.0043, 0.0038 and 0.0031 mg/L and LC$_{90}$ 0.0155, 0.0154 and 0.0140 mg/L after 24, 48 and 72 hrs. The LC$_{50}$ for 15°C was 0.0040, 0.0039 and 0.0036 mg/L and LC$_{90}$ 0.0157, 0.0182 and 0.0176 mg/L after 24, 48 and 72 hrs. The LC$_{50}$ for 20°C was 0.0028, 0.0025 and 0.0019 mg/L and LC$_{90}$ 0.0069, 0.0067 and 0.0062 mg/L after 24, 48 and 72 hrs. The LC$_{50}$ for 35°C was 0.0055, 0.0048 and 0.0038 mg/L and LC$_{90}$ 0.0209, 0.0167 and 0.0157 mg/L after 24, 48 and 72 hrs against anopheline larvae. The LC$_{50}$ for 10°C was 0.4386, 0.2612 and 0.1863 mg/L and LC$_{90}$ 2.513, 1.355 and 0.9477 mg/L after 24, 48 and 72 hrs. The LC$_{50}$ for 15°C was 0.2431, 0.1819 and 0.1516 mg/L and LC$_{90}$ 0.9688, 0.6187 and 0.4434 mg/L after 24, 48 and 72 hrs. The LC$_{50}$ for 20°C was 0.0031, 0.0017 and 0.0015 mg/L and LC$_{90}$ 0.0073, 0.0040 and 0.0037 mg/L after 24, 48
and 72 hrs. The LC$_{50}$ for 35°C was 0.0096, 0.0055 and 0.0036 mg/L and LC$_{90}$ 0.0586, 0.0309 and 0.0161 mg/L after 24, 48 and 72 hrs against culicine larvae.

Further, the most potent encapsulated nano-pesticide was tested against aquatic non-targets, *Daphnia magna* and *Cypris sps.* The results shows the LC$_{50}$ with 18.084, 16.464 and 10.990 mg/L and LC$_{90}$ 139.830, 126.006 and 92.223 mg/L after 24, 48 and 72 hrs against *D. magna* and LC$_{50}$ with 20.038, 17.336 and 12.034 mg/L and LC$_{90}$ 142.626, 122.903 and 93.727 mg/L after 24, 48 and 72 hrs against *Cypris*.

The present study revealed that the temephos was the most potent among non-capsulated individuals and among the encapsulated individuals PEG loaded with 8% temephos was found to be the most efficient against both larvae. Further, the screening of the most potent bipartite and tripartite combinations revealed that the bipartite (1:1) ratio of Temephos and *C. reflexa* was the most effective while among the encapsulated combinations PEG loaded with 8% Temephos and *C. reflexa* was the most potent among both mosquito larvae. It is concluded that the encapsulated nanopesticides were more efficient as compared to their non-capsulated counterparts. Thus, nano-encapsulation technique can be considered as an innovative alternative approach to combat mosquito vectors.