Assessment for larvicidal potentiality of encapsulated plant based nanopesticide against mosquito vectors

ABSTRACT

Nanotechnology has developed immensely in the last decade and was able to create many innovative materials with an enormous range of forthcoming applications. Now-a-days there is a wide range of applications of nanoparticles in medical, pharmaceutical, industrial, biotechnological and scientific fields. The synthesis of nanoparticles from the physical and chemical method, creates the environmental hazards. The use of the metallic nanoparticles i.e. Carbon (C), Silver (Ag), Copper oxide (CuO), Zinc oxide (ZnO), Iron oxide (FeO) and Titanium oxides (TiO₃, TiO₂) nanoparticles etc, can cause the derogatory effects on the plants, animals, humans and causes the high environmental deterioration. Several novel inventions of different nanoparticles and nanomaterials are capable to diminish the environmental problems. Nanopesticides develop and explore the possibility of Nanotechnology, which accentuate the concept of particle size reduction and its properties. Nano-encapsulation is another part of nanotechnology in which the pesticide is coated by a matrix and the size of the pesticide reduces up-to the nano size. Therefore, the nanoencapsulation helps to minimise the doses to get maximum effect on the target organisms.
In recent years, it is emphasized on the application of nanotechnology in insect pest management. Technologies like encapsulation and controlled release system (CRS) have, therefore, modernized the application of biocides. Nanotechnology based insecticides are devised by a number of companies. These formulations embrace nanoparticles of size 120-250 nm size range being more efficiently water soluble as compared to existing pesticides. In India, the relevance of nanotechnology in pest management has just started in the last decade. Pesticides encapsulated with nanoparticles like Karate and Matador are being lucratively developed by some companies like BASF (Germany), Monsanto (USA) and Syngenta (Switzerland). This has necessitated the need for a research of environmentally safe, biodegradable native method for the vector control.

Mosquito borne diseases are one of the world’s most health perilous problem. Numerous mosquito species belonging to genera *Aedes*, *Anopheles*, and *Culex* are the common vectors which causes various diseases like Dengue, Yellow fever, Malaria, Filariasis, Japanese encephalitis etc. Countless efforts have been developed to control the mosquitoes, firstly chemical insecticides like Dichloro Diphenyl Trichloroethane (DDT), Benzene Hexa Chloride (BHC), Melathion etc., was used to control the mosquito inhabitants, but, these chemicals causes ill effects on the environment, non-target organisms and also non-biodegradable in nature. Therefore, the green synthesis of nanoparticles using plant derivatives can be used to control the mosquito population instead of using metal and chemical nanoparticles.

Since, antiquity plant products have been exposed to display not only their pharmacological benefits, but, also for other biological properties including fungicidal,
microbial, insecticidal and pesticidal activities. Therefore, the botanical pesticides were initiated for the further research as they are effective, ecofriendly, easily biodegradable and non-toxic to non-target organisms. However, very insufficient documentation is available regarding the application of nanopesticides against vectors. Further, encapsulated plant based nanopesticide research is in infancy. The encapsulated plant based nanopesticides have the advantages as they can be easily taken by target organisms, more active in action as compared to synthetic nanopesticides. Plant based nanopesticides are eco-friendly being biodegradable. Many researchers pioneered that the pests fail to develop resistance against the crude plant based pesticides. They are economical being required in small quantity. Pests and vectors were unsuccessful to develop resistance against plant based nanopesticides. Nanoencapsulated pesticides are with control release have long shelf life and causing less pollution.

As compared to synthetic based nanopesticides, the encapsulated plant based nanopesticides attract extra attention and sincere efforts should be made for the development of plant based nanopesticides. In present investigation, the bioefficacy of the different plant extracts were tested and recognized the most efficient plant extract. The effect of physico-chemical parameters like light, temperature and potentia hydrogenii (pH) were thoroughly observed to ensure the most appropriate environmental conditions for the activity of the nanopesticide. The enhancement of the toxicity of nanopesticide and the retortion of treated mosquito larvae was observed. The effects on the non-targeted organisms were experimented to endorse the lethal effect of nanopesticide on the other aquatic organisms. The expiration of the phyto-nano-pesticide or bio-nano-pesticides was
also studied by tested the mosquito larvae after every 6 months of time interval and observed the shelf-life.

**The plants,** *Allium sativum* (Garlic) belonging to family Liliaceae and *Pseudocalymma alliaceum* (Garlic creeper) belonging to family Bignoniaceae were selected for present investigation. The leaves of *Pseudocalymma alliaceum* were collected from the botanical garden of the Dayalbagh Educational Institute, Agra and the cloves of *Allium sativum* from the local market. The leaves of *P. alliaceum* were washed with running tap water and dried in shade. The covers of cloves of *A. sativum* were peeled and finally dried in shade. The completely dried leaves and cloves were crushed and then stored in glass jars for further use.

**The extraction of the weighed and dried plant materials** was conducted separately in petroleum ether, hexane, and methanol successively in a Soxhlet Apparatus for 48-72 hours. The solvent selected covered the intact range (lower to higher) of polarity from petroleum ether to methanol so as to extract the maximum number of components from the leaves and cloves. Extracts were separated from the solvents by Vacuum Rotary Evaporator to get pure residues which were finally weighed and stored in refrigerator under 4-5 °C for further use.

**For the extraction of essential oil** of the fresh cloves of *Allium sativum* (garlic) and fresh leaves of *Pseudocalymma alliaceum* (garlic vine) were subjected to hydro-distillation in Clevenger type apparatus for eight hours to obtained essential oils. In the garlic vine, the oil content was in the trace amount. Therefore, the garlic oil thus obtained
was dehydrated over anhydrous magnesium sulphate to extract the oil and stored at room temperature for further use.

The target organism, 3rd instar larvae of *Anopheles stephensi* and *Culex quinquefasciatus* were selected. The mosquitoes were reared in our laboratory under the control conditions of 28 ± 2°C temperature, 75 ± 5% relative humidity.

The selection of encapsulating or coating material; Polyethylene glycol (PEG) polymer was used as a carrier loaded with garlic essential oil and was prepared by using the melt-dispersion method (Peng et al., 2008).

In the preparation of nanoparticles the essential oil of *Allium sativum* was mixed separately in different quantities with melted PEG and stirred to ensure even distribution of the mixture. 1%, 2%, 4% and 8% mixtures of essential oil of *Allium sativum* and PEG were prepared. The mixture was grounded in a mortar box and allows cooling naturally at 25°C and then sieved by using a sieve mesh-200 (pore size). The powder obtained is placed in airtight, self-scalable polyethylene pouches and stored at 25°C in desiccators containing calcium chloride to prevent moisture absorption prior to further use.

The stock solutions of *Allium sativum, Pseudocalymma alliaceum* and *Allium sativum* (garlic) essential oil were prepared independently by dissolving them in ethyl alcohol separately. A range of different desired test concentrations were prepared by dilution of stock solutions independently in 500 ml capacity of glass beakers in triplicates to expose the mosquito larvae.
In bioassay, twenty 3\textsuperscript{rd} instar culicine and anopheline larvae after 24 hours of acclimatization in lab condition were exposed to each working concentration. Three replicates were arranged with control in parallel. Mortality counts were made after 24 and 48 hours of exposure period, respectively. The experiments were set according to WHO Standard Procedure (1975). Bioassay tests showing more than 20\% mortality in control was discarded and on less than 20\%, values were corrected by the application of Abott’s formula (Abbot, 1925). LC\textsubscript{50} and LC\textsubscript{90} values were calculated by Probit analysis (Finney, 1971). The complete procedure was repeated in each extract of the selected plant material.

The separation of the compounds present in the most potent extract done by using column chromatography and the peaks of the compounds were studied by High Performance Liquid Chromatography (HPLC). This helps to authenticate the quantity and the presence of the number of compounds present in the extract.

The characterization of nanoparticles; The average size of PEG coated nanoparticles were measured by using a Zetasizer (model Nano-ZS), Malvern Instruments (U.K.). This instrument was used to verify the range of the nanoparticles present in the solution. The transmission electron microscopy (TEM) was also done to characterize the size, shape and structure of the nanoparticles used in the experiments.

The bioefficacy of different extracts of \textit{Pseudocalymma alliaceum} leaves, cloves of \textit{Allium sativum}, non-encapsulated essential oil of \textit{Allium sativum} and encapsulated oil of \textit{Allium sativum} against \textit{An. stephensi}. The LC\textsubscript{50} values of petroleum ether, hexane and methanol extracts were studied and hexane extract was found the most potent among the other extracts with LC\textsubscript{50} values 8.65 ppm and 7.49 ppm of \textit{P. alliaceum}.
and 10.82 and 7.65 ppm of *A. sativum*. The hexane extract of *Pseudocalymma alliaceum* leaves was the most effective as compared to *Allium sativum* extracts. Therefore, the separation of the different compounds present in the hexane extract of *P. alliaceum* was fractionated by using the column chromatography. Six (VI) fractions were separated from the extract. The fraction-II (F-II) was the most efficient with LC$_{50}$ values 1.85 and 0.91 ppm against the *An. stephensi* after 24 and 48 hrs of exposure, respectively. The LC$_{50}$ values of the non-encapsulated essential oil of *Allium sativum* cloves against *An. stephensi* were 1.21 and 0.35 ppm after 24 and 48 hrs of treatment, accordingly. The LC$_{50}$ values of the 2% encapsulated oil of *Allium sativum* cloves against *An. stephensi* were 14.85 (2%= 0.29) and 5.95 (2%= 0.12) ppm after 96 and 120 hrs of exposure, respectively.

The LC$_{50}$ values of petroleum ether, hexane and methanol extracts were studied against *Cx. quinquefasciatus* larvae and hexane extract of *P. alliaceum* was found the most potent with LC$_{50}$ values 2.49 and 1.16 ppm and the petroleum ether extract of *A. sativum* was the most effective as compared to the other tested extracts with LC$_{50}$ values 8.38 and 7.28 ppm after 24 and 48 hrs of treatment, respectively. The F-II was again found the most efficient against culicine larvae with LC$_{50}$ values 1.40 and 0.67 ppm after 24 and 48 hrs of exposure, respectively. The LC$_{50}$ values of the essential oil of *Allium sativum* cloves were 1.30 and 1.01 ppm after 24 and 48 hrs of exposure respectively. The LC$_{50}$ values of the 2% encapsulated oil of *Allium sativum* cloves were 27.3 (2%= 0.55) and 11.09 (2%= 0.22) ppm against the *Cx. quinquefasciatus* after 96 and 120 hrs of treatment, respectively.
In the qualitative analysis of fraction (F) II, different groups were tested like alkaloids, terpenoids, phenols, tannins, fixed oils, saponins, glycosides and flavanoids. Among these tested groups the F-II responded on the Lead acetate test which indicates the presence of tannins moiety in the fraction of hexane extract of *P. alliaceum* leaves.

The effect of the physico-chemical parameters (light, potentia hydrogenii (pH) and temperature) on the potentiality of non-encapsulated essential oil and 2% encapsulated oil of *Allium sativum* was tested to identify the most effective physical parameters against *An. stephensi* and *Cx. quinquefasciatus*.

For the effect of light on the non-encapsulated oil of *Allium sativum* was treated with fluorescent light, dark light, UV 1hr, UV 2hr and UV 4hr and tested against both the mosquito species. In *An. stephensi* larvae the fluorescent light was the most effective with \( LC_{50} \) values 0.97 and 0.66 ppm after 24 and 48 hrs of exposure, respectively. UV 2hr was found the most potent parameter with \( LC_{50} \) values 2.15 and 0.81 ppm against *Cx. quinquefasciatus* larvae after 24 and 48 hrs of exposure, respectively. The encapsulated oil of *Allium sativum*, the dark light was found the most effective against *An. stephensi* and *Cx. quinquefasciatus* larvae with \( LC_{50} \) values 16.58 (2% = 0.33 ppm) and 5.35 (2% = 0.11 ppm) ppm and 50.16 (2% = 1.00) and 39.57 (2% = 0.79) ppm after 24 and 48 hrs of exposure, respectively.

The effect of potentia hydrogenii (pH) on the non-encapsulated essential oil of *A. sativum*, pH 6.0, 6.5, 7.5 and 8.0 were tested against both the target species. pH 6.0 was observed the most effective against the *An. stephensi* with \( LC_{50} \) values 0.67 and 0.61 ppm. The \( LC_{50} \) values being 2.79 and 1.13 ppm of pH 6.5 was the most effective against the *Cx. quinquefasciatus* larvae after 24 and 48 hrs of exposure, respectively. In case of
the encapsulated essential oil of *A. sativum*, pH 6.0 was the most effective against anopheline larvae with LC$_{50}$ values 54.33 (2%= 1.09) ppm and 13.18 (2%= 0.26) ppm and the LC$_{50}$ values of culicine larvae were 36.84 (2%= 0.74) and 27.79 (2%= 0.56) ppm after 24 and 48 hrs of exposure, respectively.

The effect of the temperature the experiments were conducted at 10 °C, 15 °C, 20 °C and 35 °C for both the non-encapsulated and encapsulated essential oil of *A. sativum*. The non-encapsulated oil was found to be the most effective at temperature 10 °C, the LC$_{50}$ values 1.04 and 0.66 ppm against *An. stephensi* and at 15 °C the LC$_{50}$ values 0.33 and 0.30 ppm against *Cx. quinquefasciatus* larvae after 24 and 48 hrs of exposure, respectively. The encapsulated essential oil of *A. sativum* was observed the most efficient at temperature 15 °C, the LC$_{50}$ values 60.16 (2%= 1.20) and 32.26 (2%= 0.65) ppm against the *An. stephensi* and 10 °C the LC$_{50}$ values 150.3 (2%= 3.01) and 88.19 (2%= 1.76) ppm against *Cx. quinquefasciatus* larvae after 24 and 48 hrs of treatment, respectively, as compared to the other tested temperatures.

All these above important extrinsic factor affecting parameters studied, the pH 6.0 and temperature 15 °C was the most effective parameters as compared to the other tested parameters against the *An. stephensi* and *Cx. quinquefasciatus* larvae in non-encapsulated essential oil of *A. sativum*. The dark light and the potentia hydrogenii (pH) is the also effective in the bioactivity of 2% of encapsulated oil of *Allium sativum* against anopheline and culicine larvae.

**In the bioefficacy of non-target organism** *Daphnia* and *Cipris* were also tested by 2% encapsulated *Allium sativum* oil. The LC$_{50}$ values were 486.5 (2%= 9.73) and
255.1 (2% = 5.10) ppm against *Daphnia* and the LC$_{50}$ values were 575.1 (2% = 11.5) and 400.9 (2% = 8.02) ppm against *Cipris* after 24 and 48 hrs of exposure, respectively.

In the developmental experiments of both the species of mosquito larvae, the encapsulated oil of *Allium sativum* was originated as the efficient bio-nano-pesticide. In the present investigation, the development of the *Anopheles stephensi* and *Culex quinquefasciatus* egg to adult was observed. In anopheline development, the hatching of the larvae was partially hampered in low concentrations, however, in later stages development was arrested after larval stage. In control the larvae transformed into the pupae. The adult transformation does not take place in treated and the control experiments. In culicine mosquito, the hatching of the larvae decreases as the concentration increases. In lower concentrations, the hatching was 100% and the larval development was arrested till the 17$^{th}$ day of the experiment conducted. The pupal transformation was not observed up to the 17$^{th}$ day and the mortality of the larvae observed in the treated sets of experiment. In the control set of experiment, the complete development from egg to adult was noted in the culicine mosquito eggs.

**In the morphometric studies,** the anopheline and culicine eggs were distorted, the structural damage was marked by loss of bristles, alimentary canal was ruptured, disorganization of haemolymphatic tissues and loosening of arthropodal joints were observed in anopheline and culicine larvae. In the morphology of non-target organisms, the structure and the body tegument were not damaged. The organs were also not degenerated or distorted in the target organisms (*Daphnia* and *Cypris*).
It is concluded that the 2% encapsulated oil of *Allium sativum* cloves was found the most effective as compared to other experimented extracts against the *Anopheles stephensi* and *Culex quinquefasciatus* mosquito larvae. The encapsulated oil was more effective during dark light and at pH 6.0 as compared to non-encapsulated oil. It was also observed that the encapsulated oil arrested the development and the morphological stages of the anopheline and culicine mosquito. The bionanopesticide reduces the hatching percentage of the larvae. Therefore, the development in both mosquito species was arrested owing to the slow and controlled release of the encapsulated oil. The encapsulated oil was very effective and it does not influence the non-target organisms (*Daphnia* and *Cypris*). The encapsulated oil has enhanced shelf life. The arrested development at larval stage maintains the food-chain for the other larvivorous aquatic organisms and thus maintains the natural environment & bio-diversity. Thus, the encapsulated *Allium sativum* essential oil based nanopesticide being ecofriendly & economical is an ideal larvicide against *Anopheles* and *Culex* mosquitoes.