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Synthesis of Silver Nanoparticles from *Achyranthes aspera* Leaf Extract and its Larvicidal activity against *Aedes aegypti*

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Abstract: Mosquitoes cause more human sutterings than any other organisms, and over one million people worldwide die from mosquito-borne diseases every year. In recent years the utilization of secondary metabolites from plant extract has emerged as a novel technology for the synthesis of various nanoparticles. The aim of the present study was to evaluate the effect of plant synthesized silver nanoparticles (Ag NPs) using aqueous leaf extract of *Achyranthes aspera* to control dengue vector *Aedes aegypti*. The synthesized AgNPs were characterized by UV–vis spectrum and Fourier transform infrared spectroscopy (FTIR). Synthesized silver nanoparticles (AgNPs) particles were confirmed by analyzing the excitation of surface plasmon resonance (SPR) using UV–vis spectrophotometer at 404 nm. The FTIR analysis of the nanoparticles indicated the presence of proteins, which may be acting as capping agents around the nanoparticles. Biosynthesis of nanoparticles may be triggered by several compounds such as carbonyl groups, terpenoids, phenolics, flavonones, amines, amides, proteins, pigments, alkaloids and other reducing agents present in the biological extracts. These results suggest that the synthesized Ag NPs have the potential to be used as an ideal eco-friendly approach for the control of the *A. aegypti*.

Keywords: *Achyranthes aspera, AgNPs, Aedes aegypti,* UV, FTIR,

1. INTRODUCTION

Mosquitoes are the major vector for the transmission of Malaria, Dengue fever, Yellow fever, Filariasis, Chikungunya, *Schistasomiasis* and *Japanese encephalitis* (Gubler 1998). Mosquitoes also cause allergic responses in human that include local skin and systemic reactions such as angioedema (Peng *et al.*, 1999). *Aedes aegypti*, vector of dengue is widely distributed in the tropical and subtropical zones. Dengue fever incidence has increased fourfold since 1970 and nearly half the world's population, 1.5 billion people lived in regions where the estimated risk of dengue transmission was greater than 50% (Hales *et al.*, 2002).

Ae. aegypti is considered to be a vector of dengue fever, a disease endemic to South East Asia, Africa and America (Maillard *et al.*, 1993). More than 50 Million people are at risk of dengue virus exposure worldwide. Annually, there are two million infections 5,00,000 cases of dengue *haemorrhagic* fever and 12,000 deaths (Kovendan *et al.*, 2012). Although yellow fever has been reasonably brought under control with its vaccine, no vaccine is available for dengue. The only way of decreasing the incidence of this disease is the eradication of *Ae. aegypti* (Syedali *et al.*, 2012). *Aedes* mosquitoes on the other hand are also painful and persistent biters. *Ae. aegypti* is responsible for spreading dengue. The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion people over 40% of the world's population are now at risk from dengue. WHO currently estimates there may be 50 - 100 million dengue infections worldwide every year (WHO, 2012).

To control mosquitoes and mosquito borne disease which have world health and economic impacts, synthetic insecticides based interventions are still necessary, particularly in situations of epidemic outbreak and sudden increase of adult mosquitoes (Nathan *et al*., 2006). Insecticide application although highly efficacious against the target species, vector control is facing a threat due to the development of resistance to chemical insecticide resulting in rebounding vectorial capacity (Liu *et al*., 2006). It has also provoked undesirable effects, including toxicity to non-target organisms and fostered environmental and human health concerns (Yang *et al*., 2002). So, there is an urgent need to develop new insecticides for controlling mosquitoes which are more environmentally safe and also biodegradable and target specific against vectors/parasites.

Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environmental factors. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities (Shaalan *et al.,* 2005). Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction. A wide selection of plants from herbs, shrubs and large trees was used for extraction of mosquito toxins. Phytochemicals were extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks, roots, *etc*., of larger plants or trees. In all cases where the most toxic substances were concentrated upon, found and extracted for mosquito control.

Plants produce numerous chemicals, many of which have medicinal and pesticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes. Members of the plant families- Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have various types of larval, adulticidal or repellent activities against different species of mosquitoes (Shaalan *et al.,* 2005). In addition to the direct use of phytoextracts, in recent days, biosynthesized silver nanoparticles gain momentum as biocontrol agents against mosquitoes and microbes. In these circumstances, an improvised method using the biologically synthesized silver nanoparticles were evaluated for the destruction of the mosquito larvae *Ae. albopictus*. Silver nanoparticles are emerging as one of the fastest growing material due to their unique physical, chemical and biological properties, small size and high specific surface area (Sarah *et al*., 2012).

Vivek *et al.,* (2011) have reported the synthesis of bio nanoparticles using *Sargassum wightii, Kappaphycus alvarezii* and *Gelidiella acerosa* crude extract respectively. Seaweeds have various phytochemicals including Carbohydrates, Alkaoids, Steroids, Phenols, Saponins and Flavonoids act as a reducing agents of silver. A rapid reduction of the silver ions occurred when the silver nitrate solution was contacted with geranium leaf extract. Studies have indicated that biomolecules like protein, phenols and falvonoids not only play in reducing the ions to the nano sizes, but also play an important role in the capping of the nanoparticles (Raja *et al*., 2012). India being rich in herbs can utilize its herbs for such purpose, plants not only being pesticides and insecticides it can also act as an effective antimicrobial, antifungal, antiparasitic and anti-malarial agents.

Thus it has being expensively exploited for these properties, here in the present study we have used the plant source *Achyranthes aspera* and Ag NPs on Larvicidal activity against *Ae. Aegypti*.

2. MATERIALS AND METHODS

2.1 Study material: *Achyranthes aspera* a leaf (Fig.1) extract was chosen for the present study.

2.2 Sample collection: *A. aspera* was collected from Pullalakkottai village in Viruthunagar (Dist). The plant was collected and washed several times with tap water to remove dust and soil. The leaves were removed and it was washed with tap water and rinsed with distilled water. The cleaned

leaf material was dried in shade at room temperature and stored for further use.

Fig.1: *Achyranthes aspera leaf*

2.3 Preparation of acetone extract: The dried leaves were used to prepare the extract adopting the procedure described by Satyavani *et al*. (2011). 25gms of the dried material was powdered mechanically using electrical stainless steel blender. The powder was mixed with 250ml acetone and boiled (boiling point range *55.5-56.5*^{0}C) in Soxhlet apparatus for 8hrs. The extract collected was stored at 4^{0}C for further use.

2.4 Synthesis and purification of silver nanoparticles: For synthesis of silver nanoparticles, 10ml acetone extract of *A. aspera* was added to 90ml 1mM solution of silver nitrate in 250ml conical flask and kept at room temperature for 1hr. The primary detection of synthesized silver nanoparticle was carried out in the reaction mixture by observing the colour change of the medium. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 5,000rpm for 20mts. The supernatant was discarded and the pellet was dissolved in double distilled water. The silver nanoparticles were confirmed by colour change (Satyavani *et al*., 2011).

2.5 Characterization of silver nanoparticles: The produced nanoparticles were subjected to UV-Vis spectroscopy analysis, and Fourier Transform Infrared Radiation (FTIR) spectroscopy analysis at Madurai Kamaraj University.

2.5.1 UV-Vis absorbance spectroscopy analysis: The samples used for analysis were diluted with 2ml of double distilled water and subsequently measured by the UV-Vis spectroscopy at regular time intervals by using a quartz cuvette with water as a reference (Rajesh *et al*., 2009), UV-Vis spectroscopy analysis of silver nanoparticles produced were carried out as a function of bioreduction time at room temperature on ELICO spectrophotometer at a resolution of 1nm. The UV-Vis spectrometric readings were recorded at a scanning speed of 200-800nm.

2.5.2 Fourier transform infrared radiation spectroscopy analysis: To remove free biomass residue (or) compound that is not the capping ligand of the nanoparticles, the residue solution of 100ml after reaction was centrifuged at 5,000rpm for 10mts. The supernatant was again centrifuged at 10,000rpm or 60mts, and the pellet was obtained. This was followed by redispersal of silver nanoparticle pellets into 1 ml deionized water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried silver nanoparticles were analyzed by FTIR analysis (Vivek *et al*., 2011).

2.6 Larvicidal bioassay: The eggs of *Ae. aegypti* was procured from Centre for Research in Medical Entomology (ICMR), Madurai, India. The larvae were kept in enamel tray containing distilled water. They were maintained and reared in the laboratory as per method adopted by Kamaraj *et al*. (2009) and all experiments were carried out at room temperature. Larvae were fed with yeast powder and dog biscuits. The larvicidal activity was assessed by following the procedure of WHO (1996) with modifications as per the method described by Rahuman *et al*. (2000). Twenty five number of 3rd instar larvae of *Ae. aegypti* were transferred separately from culture being maintained in the laboratory to the 250 ml beaker containing the 100 ml of desired concentration of plant extracts and AgNPs. The control was set up with dechlorinated tap water. The Moribund larvae were counted after 24h of exposure and the percentage mortality was recorded for the average of four replicates.

2.7 Statistical analysis: Statistical analysis such as LC_{50}, 95% confidential limit and chi square values were calculated by using EPA Probit analysis programme version 1.5.

3. RESULTS

3.1 Synthesis of Silver Nanoparticles: The change in colour was noted by visual observation in the silver nitrate solution incubated with *A. aspera* extract (Fig.2). The colour of the extract was changed to reddish brown in 1hrs at 37^{0}C due to the formation of silver nanoparticle by the reduction of the silver metal ions by the extract of *A. aspera*. Control sample silver nitrate without *A. aspera* extract did not show any change in colour.

Fig.2: Colour change during the bioreduction of $AgNO_3$ into AgNPs using plant extract: before synthesis (left) and after synthesis (right).

3.2 Spectral characterization

3.2.1 UV-Vis spectrum of silver nanoparticles: Reduction of silver ions in the aqueous solution of silver during the reaction with the ingredients present in the plant leaf extract was observed by the UV-Vis spectroscopy. The change in colour was noted by visual observation in the *A. aspera* leaf extract when it was incubated with $AgNO_3$ solution. *A. aspera* extract without $AgNO_3$ did not show any change in colour. The colour of the extract changed to light brown within an hour and then later changed to dark brown during the 2hrs incubation period. No significant change occurred after 2hrs. The brown colour could be due to the excitation of surface plasmon vibrations, typical of the silver nanoparticles (Krishnaraj *et al.*, 2010). In the present study, UV–Vis spectra of leaf solution as a function of reaction time. The strong resonance centered at 404 nm was clearly observed and increased in intensity with time. It might arise from the excitation of longitudinal plasmon vibrations in AgNPs in the solution. *A. aspera* leaf solvent (Acetone) extract was subjected to synthesis of Silver nanoparticles and the visible colour change (Fig.2) indicates the formation of nanoparticles which is confirmed by UV-Visible absorption spectroscopy. The progress of the reaction between metal ions and the leaf extracts were monitored by UV– visible spectra of silver nanoparticles in aqueous solution with different reaction times that are shown (Fig.3).

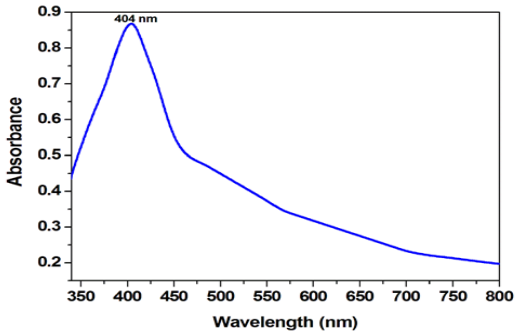

Fig. 3: UV-Vis spectra of silver nanoparticles synthesized by treating *Achyranthes aspera* leaf extract with 1mM silver nitrate solution.

It was observed from the figure that the peak blue shifted in the absorption spectrum from 425 nm with increasing reaction time from 30 min to 120min. It took two hours to complete the reaction to form stable nanoparticles.

3.2.2 Fourier Transform Infrared Radiation Spectroscopy Analysis: FTIR measurements were carried out to identify the potential bio molecules in the leaf extract responsible for the reduction and also the capping reagent responsible for the stability of the bio reduced silver nanoparticles. From the leaf extract, the absorption bands at 3396.76cm^1, 1647.26cm^{-1}, 1454.38cm^{-1}, 1406.15cm^{-1}, 1112.96cm^{-1} and 663.53 cm^{-1} were recorded. Whereas the intense band in AgNPs spectrum at 3396.76cm^{-1}corresponds to O-H stretching and the band at 1647.26cm^{-1} corresponds to amide I, arising due to carbonyl stretch in proteins (Fig.4).

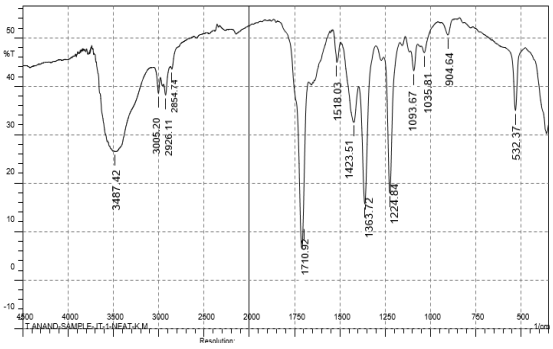

Fig.4: FTIR spectrum of biosynthesized AgNPs

The weak band at 663.53cm^{-1}is –C-O- stretch. It is observed from the spectra of Ag nanoparticles the appeared bands at 3396.76cm^{-1} and 1647.26cm^{-1}which are due to hydroxyl group and amide-I are

responsible for reducing the Ag ions to atoms and suppressed bands at 1454.38cm^{-1}, 1406.15cm^{-1} and 1112.96cm^{-1} are responsible for stabilizing the nanoparticles. The peaks at 663.53cm^{-1} indicate the presence of alkyl halides. These records indicate the presence of silver ions formed due to the bioreduction and their stabilization as nanoparticles. The results revealed that the capping ligand of the AgNPs may be hydroxyl (-OH), Benzene ring, carboxylic acid (C=O) and alkyl halides.

3.3 Larvicidal activity of leaf extract and synthesized silver nanoparticles: In the present study was carried out to establish the larvicidal activity of synthesized silver nanoparticles (AgNPs) using leaf extract of *A. aspera* against the third instar larvae of *Ae. aegypti* was determined (Table 1 & 2). The synthesized AgNPs from *A. aspera* were highly toxic than leaf solvent extract in both mosquito species. The LC_{50} values of synthesized AgNPs against third instar larvae of *Ae. aegypti* within 24h, 48h and 72 h (8.37 ppm, 5.42 ppm, 4.08 ppm) respectively. The LC_{50} values of leaf extract of *A. aspera* against third instar larvae of *Ae. aegypti* within 24h, 48h and 72 h were 96.36 ppm, 75.98 ppm, 56.05 ppm.

Table 1: Larvicidal activity of leaf extract of synthesized silver nanoparticles of *Achyranthes aspera* against *Ae. aegypti*

Time Duration	LC_{50} value (ppm)	95% Fiducial limit		Chi-square χ^2	S.E	Slope
		LFL	UFL			
24 hours	8.370	7.415	9.447	5.422	0.392	3.322
48 hours	5.424	4.091	7.192	15.006	0.494	3.238
72 hours	4.082	2.835	5.879	19.406	0.504	3.050

LFL- Lower Fiducial Limit UFL - Upper Fiducial Limit

Table 2: Larvicidal activity of leaf solvent extract of *Achyranthes aspera* against *Ae. aegypti*

Time Duration	LC_{50} value (ppm)	95% Fiducial limit		Chi-square χ^2	S.E	Slope
		LFL	UFL			
24 hours	96.361	87.072	106.641	7.721	0.451	2.529
48 hours	75.987	58.086	99.380	16.837	0.515	3.091
72 hours	56.055	39.294	79.965	21.353	0.486	3.567

LFL- Lower Fiducial Limit UFL - Upper Fiducial Limit

4. DISCUSSION

Vector borne disease constitutes the major cause of morbidity in most of the tropical and subtropical countries and has always been a challenge to the medical professionals struggling for the welfare of humanity. Recent figures from the World Health Organization (WHO) evidenced that the prevalence of dengue fever has increased over 50 years and 2 billion people are under risk in more than 100 centuries. In 2007, incurable crippling disease chikungunya, spread primarily by *Ae. aegypti* sps has resurfaced in Kerala, Southern state of India. Many plants derived natural compound was tested for mosquito control (Chakkaravarthy *et al.*, 2011). Silver has been used for many years for its antimicrobial properties. Alexander the Great used silver vessel to store drinking water (Silver *et al.*, 2006). However, the formulation of silver has changed during antiquity from bulk silver to ionic silver (Kwakye-Awuah *et al.*, 2008). The broad spectrum antimicrobial properties of silver nanoparticles encourage its use in a large number of biomedical and environment applications as well as in growing list

cosmetics, clothing, and numerous consumer products (Jain *et al*., 2011

Metal nanoparticles can be synthesized by reducing metal ion using some chemicals. In biosynthesis, it is believed that extracts of natural materials act as reducing agent for generation of metal nanoparticles (Monavallil *et al*., 2010). Previous studies have assured that the reduction of the silver ions into silver nanoparticles during exposure to the sea weed extracts of *Gelidiella* sp could be followed by colour change. AgNPs exhibit dark yellowish brown colour in solution. Absorption spectra of silver nanoparticles formed in reaction media at 72hrs (Bhima *et al*., 2012). The present study demonstrated the formation of the silver nanoparticles by the reduction of the aqueous silver metal ions during exposure to the extract of *A. aspera*. The reaction of ions occurred within 1hr at 37^0C and appearance of reddish brown colour from colourless solution.

Silver nanoparticles synthesized by using *A. aspera* were formed at 404 nm with polydispersed. UV-Vis spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles (Sun *et al*., 2001). Generally, UV-VIS spectroscopy can be used to examine size and shape of the controlled nano particles in aqueous suspense. The results of the UV-VIS absorption showed increasing colour intensity with increased time intervals and this might be due to the production of the silver nanoparticle (Shrivastava *et al*., 2010) and the formation of the brownish yellow colour might be due to the excitation of the surface plasmon vibration of the synthesized silver nanoparticles (Krishnaraj *et al*., 2010).The broadness of the peak is a good indicator of the size of the nanoparticles. As the particle size increases the peak becomes narrower with a decreased bandwidth (Kong and Jang., 2006). Jain *et al*. (2011) have reported that the absorption spectra of silver nanoparticles were highly symmetric single band absorption with peak at 421nm.

FTIR analysis was carried out, to identify the functional groups of the synthesized AgNPs. FTIR spectrum indicated the clear peaks with (3396.76cm^{-1}, 1647.26cm^{-1}, 1454.38cm^{-1}, 1406.15cm^{-1}, 1112.96cm^{-1} and 663.53 cm^{-1}) different values (Fig. 4). Above the peak values they corresponded to functional groups like, amide group (N–H stretching 3396.76cm^{-1}), an aliphatic group (cyclic CH2 – 2947.33cm^{-1}), a methyl group (bend CH2–CH3 stretching 1454.38cm^{-1}), aliphatic amine group (C–N stretching 1026.16cm^{-1}), alkyl halides group and alkyl halides (C–Br stretching 663.53 cm^{-1}). The functional groups such as alcohol, amines, amides, alkanes, methyl, aliphatic and halides confirmed their presence in AgNPs and these are the main classes in most of the functional groups. They were denoted as possible biomolecules responsible for stabilizing, capping and reducing agents of the AgNPs (Vijayaraghavan *et al*., 2012; Srinivasan *et al*., 2014). The terpenoid groups have a high potential to convert the aldehyde groups into carboxylic acids in the Ag+ medium. Additionally, amide groups are also responsible for the presence of the some enzymes which, may be responsible for the synthesis of metal particles. Further, polyphenols are also proving that they have the potential to reduce the silver metals (Srinivasan *et al*., 2014).

In present investigation, third instar larvae of *Ae. aegypti* was treated with biosynthesized silver nanoparticles and the percent mortality was assessed against various concentrations ranging from 2-10ppm. The LC_{50} value of synthesized silver nanoparticles was 8.370ppm. The mechanism which causes the death of the larvae could be explained due to the nanoparticles can enter through oral cavity as well as body membrane of the Mosquito larvae. These AgNPs in the intracellular space can bind to sulphur containing proteins or to phosphorus containing compounds like DNA, leading to the denaturation of some organelles and enzymes, subsequently, the decrease in membrane permeability and disturbance in proton motive force causes loss of cellular function and finally cell death. Moreover in the present case, it is evident that the active compounds phytosterols, flavonoids, Glycosides, Alkaloids, Tannins, Saponin's and Triterpenoids which are having high larvicidal activity and also contributing towards the high mortality rates with less concentration of AgNPs. Sakulku *et al*. (2009) have reported the low release rate of nano emulsion with large droplets size that resulted in prolonged mosquito repellent activity compared to the

nano emulsion with small droplets. The results of larvicidal activity clearly indicates that the percentage of mortality being directly proportional to concentration of the extract. After exposure to the test concentrations, the treated larvae exhibited restlessness, sluggishness, tremors, and convulsions followed by paralysis at the bottom of the bowl. The larvicidal effect of aqueous crude leaf extracts, silver nitrate, and synthesized silver nanoparticles of *Mimosa pudica* showed that the highest mortality was found in synthesized AgNPs against the larvae of *Anopheles subpictus* (LC50=8.89, 11.82, and 0.69 ppm) and against the larvae of *Culex quinquefasciatus* (LC50=9.51, 13.65, and 1.10 ppm) (Marimuthu *et al.,* 2011). To our best of knowledge there is no report in the literature for the control of mosquito population by using *A. aspera*. This is an ideal eco-friendly approach for the control of against dengue vector *Ae. aegypti*.

Sarah *et al.,* (2012) reported that silver nanoparticles synthesized from aqueous leaf extract of *Hibiscus rosasinensis* against the larvae of *Ae. albopictus* mosquito showed high larval mortality compared to aqueous extract and subsequently, the decrease in membrane permeability and disturbance in proton motive force causes loss of cellular function and finally cell death. In the present study acetone extract of *A. aspera* was tested for its efficiency against third instar larvae of *Ae. aegypti*. The larvae were subjected to different concentrations (20-100ppm) of the leaf extract 96.361ppm was recorded as LC_{50} value for *Ae. aegypti*. Aqueous (Physiological saline) extract of seed kernel from soap nut *Sapindus emarginatus* (Sapindaceae) was found to exhibit, a strong anti-mosquito activity as evident from its ability to inflict 100% mortality of all the developmental stage of *Ae. aegypti* (Koodalingam *et al.*, 2009).

In the present study, the biosynthesised silver nanoparticles from leaf extract of *A. aspera* showed potential larvicidal activity against *Ae. aegypti*. Hence, the larvicidal activity of the silver nanoparticles might be due to the denaturation of the sulfur-containing proteins or phosphorous containing compound like DNA that, leads to the denaturation of organelles and enzymes (Choi *et al.,* 2008) and thus reduces the cellular membrane permeability and reduction in ATP synthesis which finally causes the loss of the cellular function and cell death (Sap-Iam *et al.,* 2010). The formed silver nanoparticles are characterized by UV-Vis, and FTIR analysis; these silver nanoparticles are highly stable and had significant mosquito larvicidal activity against the third instar larvae of *Ae. aegypti*.

5. CONCLUSION

The presented green synthesis shows that the environmentally benign and renewable source of *A.aspera* be used as an effective reducing agent for the synthesis of AgNPs. This biological reduction of metal would be boon for the development of clean, nontoxic, and environmentally acceptable green approach to produce metal nanoparticles, involving organisms even ranging to higher plants. The formed Ag NPs are highly stable and have significant larvicidal activity. The prospect of utilizing plant based products for synthesizing silver nanoparticles and testing its efficacy in controlling mosquitoes as larvicides is a recent phenomenon facilitating the development of a more potent and eco-friendly pesticide. Identification of the bioactive principles involved and their mode of action and field trials are necessary to recommend an effective formulation as an anti-mosquito control programmes.

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