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Original Research Article

Larvicidal Activity of Five Different Plant Extracts against Aedes Aegypti (Linn.)

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ABSTRACT

Mosquitoes act as the major vectors for many human diseases. More recently the incidence of chikungunya is increasing steadily in different parts of Tamil Nadu has also been reported on a regular basis. Due to the insecticidal contamination of the environment and to the physiological and behavioral resistance of vectors, increasing attention is now being focused on biological control methods. Crude plant extracts are more cost effective in community based control programs and may be employed in localized situations where mosquitoes have become tolerant to synthetic or microbial larvicides.

Five different plant extracts were *Allium sativum* (Garlic bulb extract), *pongamia pinnata* (pongam oil extract) *Madhuca indica* (Illuppai oil extract), *Azadirachta Indica* (Neem oil extract), *Ocimum sanctum* (Tulsi Leaf extract) were selected and tested against early fourth instars larvae of *Ae.aegypti* and probit analysis was made to calculate the Lc_{50} and Lc_{90} values. The larvae of *Ae. aegypti* treated with five different plant extracts and the toxicity (Lc_{50} and Lc_{90}) of the larvae showed a remarkable variations. The five different plant extracts showed an effective larvicidal activity against *Ae.aegypti.* Among the five plant extracts tested, *Allium sativum* was more effective than the other plant extracts. *Ocimum sanctum* has least larvicidal effect when compared with the four plant extracts.

Therefore judicious application of the above plant extracts in the breeding medium will reduce the population of *Aedes aegypti* in an ecofriendly manner and in turn it reduces the incidence of chikungunya and various dreadful diseases in an ecofriendly manner.

Key words: Mosquito larva, plant extracts, larvicidal activity, Ecofriendly tool.

INTRODUCTION

Mosquitoes are pestiferous insects, which are responsible for the transmission of various dreadful diseases and nuisance to human beings. Mosquitoes are highly adapted insects and co-exist with human all the time, feeding on them and always fight with them. They are found all over the world particularly in tropical climates. Abundantly they are found in Marshy lands, near collected waters and stagnant ponds. Mosquitoes belong to the order diptera and all the 2500 species described so far belong to the family culicidae. The four important groups of mosquitoes in India which are related in disease transmission are the Anopheles, Culex, Aedes and Mansonia (Fenemore P.G and Alka Prakash, 2000).

Aedes aegypti occupies a very special position in preventive medicine. It is the first proved vector of viral disease such as yellow fever, filariasis, chikungunya and encephalitis (Dorland,

1982, Mazzari and Georhious, 1995). These Mosquitoes are also capable of transmitting dreadly dengue viruses (DENI, DEN2 &DEN3) (Lourenco-de-oliveira *et al.,* 2002).

Chikungunya is an arthropod-borne oral infection resembling dengue caused by group IV RNA arbovirus and transmitted by *Aedes aegypti* mosquito, which presents with symptoms of fever and joint pain. The earliest reported outbreak of Chikungunya in India was in 1963-64. The present outbreak in India started during December 2005, where the country has so far experienced more than 11, 00,000 of Chikungunya infected cases which still continues.

Aedes aegypti is the most important vector, other species such as *Ae. albopictus, Ae. pdynesiensis, Ae. suctellaris complex* and *Ae. niveus* have also been incriminated as secondary vectors. Since the 1980's, yellow fever has reemerged across Africa and in South Africa. The total of 18135 yellow fever cases and 4522 deaths reported from1987 to 1991 represents the greatest amount of yellow fever activity reported to the world Health organization (WHO) for any five year since 1958 (Robertson *et al.,* 1996).

Aedes mosquitoes breed in artificial accumulations of water in and around human dwellings such as water found in discard tins, broken bottles, flower pots, coconut shells, tanks and tree holes. Aedes mosquitoes are easily distinguished by white stripes on a black body because of the striped or banded character of their legs they are sometimes referred to as "Tiger mosquitoes" (Park, 2000).

Adult female mosquitoes of *Aedes aegypti* require a diet of mammalian blood to nourish their fertilized eggs. For this reason, they have evolved with specialized physical machinery, within two or three minutes the insect fills her "tank". Fully loaded, engorged to more than twice her normal weight, she lumbers slowly into the air. The mosquito remains sluggish for some time while the blood is digested. One meal is enough to nourish 300 eggs. (Illustrated Encyclopedia of Science and Nature, 1998).

The eggs are laid in stagnant and dirty waters ponds, Pools and ditches. Aedes lays her eggs singly. The eggs are cigar-shaped and do not possess lateral floats. The eggs hatch out within 1-2 days. The period that elapses from the moment a blood meal is taken until the eggs are laid is called the 'Gonotrophic cycle'. It is about 48 hours in hot and humid tropical areas.

The larvae are called wrigglers because of their wriggling movement. They are voracious feeders, feeding on algal, fungal spores and bacteria. The larval stage occupies 5-7 days. The pupa resembles a question mark. Its tail terminates in a pair of paddles, which assist the pupa to dive rapidly with jerky motions. The pupal stage lasts for 1-2 days. Under favorable conditions of temperature and food supply the life cycle from the egg to adult is complete within 7-10 days (Nalina Sundari M.S and Santhi R, 2006).

Man is the only vertebrate host involved in the transmission and circulation of such dreadly viruses and vector is generally *Aedes aegypti.* This vector maintains a selective pressure increasing the transmission of virus capable of producing high viremia in man (Digoutte, 1999)

Most of the mosquito control programmes target the larval stage in their breeding sites with larvicides, because adulticides may only reduce the adult population temporarily.

Mosquitoes in the larval stage are attractive target for pesticides because they breed in water, and thus are easy to deal with them in this habitat. The use of conventional chemical pesticides has resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concerns.

It is necessary to apply appropriate methods to control the mosquito populations at the larval stage. There are several methods found to be effective in recent times due to development of behavioural and physiological resistance exhibited by the mosquitoes. The development of resistance to several insecticides has led to the search for an alternative method which is sustainable and cost effective (Ravindran, 2000).

In 2008, Dua *et al.*, reported that several plants species from different families were having mosquitocidal and larvicidal activity. Three piper species have shown activity against *Ae.aegypti* (Choo-Chote *et al.*, 2006).

The widespread use of synthetic insecticides for the control of pests as well as human disease vectors has led to concerns about their toxicity and environmental impact (Bounias, 2003). Therefore it was felt to make a study on the effect of plant extracts in controlling larval population.

Plant products are easily degradable and less toxic to the environment when compared to synthetic pesticides like DDT, HCH, BHC etc. A variety of plant species of different families have been reported to exhibit insecticidal and other biological activities (Saxena and Saxena, 1992).

In this context an attempt has been made to evaluate the individual effect of plant extracts against the toxicity of larvae *Aedes aegypti* linn.

MATERIALS AND METHODS

Selected Mosquito Species: *Aedes aegypti* Linn serves as the important domestic vector of Urban yellow fever, dengue and chikungunya. It is a container breeder and found in the tropical regions. It is abundant throughout the year in Periyakulam. This mosquito species was selected for the present study.

Taxonomy:

Systemic position of *Aedes aegypti* Linn is as follows.

Kingdom	-	Animalia
Phylum	-	Arthropoda
Class	-	Insecta
Order	-	Diptera
Family	-	Culicidae
Genus	-	*Aedes*
Species	-	*aegypti*

Collection and Maintenance of Colony:

Eggs of *Aedes aegypti* were obtained from Centre for Research in Medical Entomology (CRME) at Madurai, and then incubated in plastic trays containing tap water at room temperature. After hatching the larvae fed with yeast powder and dog biscuits. The water was changed once in a day to avoid larval mortality due to microbial contamination. They were moult up to early fourth instar and used for the experiment.

Selection of Plants for the Study:

1. *Allium sativum* (Garlic) (L) (Liliaceae)

The essential oil obtained from the bulb contains allicin, diallyl disulfide, allyl prophl disulfide and other sulfur compounds.

2. *Ocimum sanctum* (Tulaci) (L) (Lamiaceae)

Eugenol, Eugenol methol, ether and carvacrol, methyl chavicol, cineole and linalool.

3. *Azadirachta indica* (Neem) (Meliaceae)

Azadirachtin, Azadiradione, Azadirone.

4. *Pongamia pinnata (*Punkai) (L) (Fabaceae)

Pongaglabrone, diketone pongamol, glabrin, karanjin, Pongapin, Kanjone

5. *Madhuca indica* (Illuppai) (Sapotaceae)

Myristic, palmitic, stearic, Arachidic, Oleic, Linoleic

(Narayan Das Prajapati *et al.*, 2004)

Preparation of Plant Extracts

Garlic Bulb Extract: Garlic bulb is crushed with water to prepare garlic extract. The Garlic bulb extract is filtered through a fine cloth and the extract obtained is used for the experiment (Nalina Sundari M.S & Santhi R, 2006).

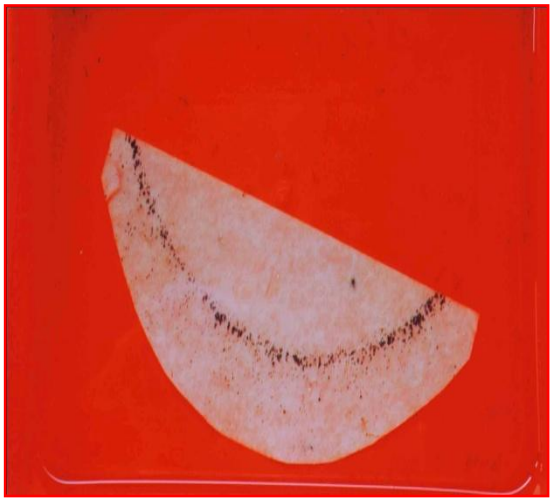

Plate 1: Egg of *Aedes aegypti*

Plate 2. Larvae of Aedes aegypti

Neem, Pongam and Illuppai Oil Extract: 20ml of each oil is mixed in one litre of water. Thin slices of khadi soap (about 5gms) are added to solution and mixed properly till white foam is produced.

Bioassay Study: Ten early fourth instar larvae of *Aedes aegypti* were separately introduced in glass beakers of 250ml capacity containing different ppm concentrations of test solutions. The test solutions were prepared with distilled water respectively in triplicates. A control set was also maintained. Mortality of the larvae was observed after 24hrs and the LC_{50} and LC_{90} values were calculated by Finney's (1971) method. The toxic effect of plant extract was confirmed by this test.

TULSI LEAF EXTRACT: The leaves should be soaked in water over night. The next day the leaves are grounded and the extract was filtered. To this 1ml of khadi soap solution is added and stirred properly. This serves as an emulsifier. (Narayan Das Prajapati *et al.*, 2004).

Plate 3. Larvae introduced in different ppm concentration of test solutions.

RESULTS

The present study has been conducted to assess the larvicidal activities of five different plant extracts.

Table1 and figure 1 shows the LC_{50} value of *Allium sativum* (Garlic bulb extract) against the early IV instar larvae of *Ae. aegypti*. The LC_{50} value was 0.4287ppm and Lc_{90} value was 0.79272 ppm.

The Lc_{50} value of *pongamia pinnata* (Pongam oil extract) against the early IV instar larvae of *Ae. aegypti* was 0.8943 ppm and Lc_{90} value was 1.1694 ppm (Table 1 and figure 2).

Table 1 and figure 3 shows the LC_{50} value of *Madhuca indica* (Illuppai oil extract) against the IV instar larvae. The LC_{50} value was 0.9532 ppm and LC_{90} value was 1.1520ppm.

The early IV instar larvae of *Ae.aegypti* on treatment with *Azadirachta indica* (Neem oil extract) the LC_{50} value was 0.9673ppm and LC_{90} value was 1.2119 ppm (Table 1).

Table: 1 Toxicity of plant extracts against larvae of *Aedes aegypti Linn*

Name of the plant extract	LC_{50} (ppm)	95% Fiducial limits		LC90 (ppm)	95% Fiducial limits		Probit regression equation
		Upper Limits	Lower limits		Upper Limits	Lower limits	
Allium sativum	0.4287	0.5233	0.3341	0.7972	0.9785	0.6159	Y=3.508469 + 3.478752x
Pongamia pinnata	0.8943	0.9140	0.8746	1.1694	1.2102	1.128	Y=0.8330102+ 4.659432x
Madhuca indica	0.9532	0.9708	0.9355	1.1520	1.1920	1.1120	Y=-1.144935 + 6.446635x
Azadirachta indica	0.9673	0.9998	0.9347	1.2119	1.260	1.1578	Y=-6.74066 + 5.238673x
Ocimum sanctum	2.4287	2.5233	2.3341	2.7972	2.9785	2.6159	Y=-3449074 + 5.238673x

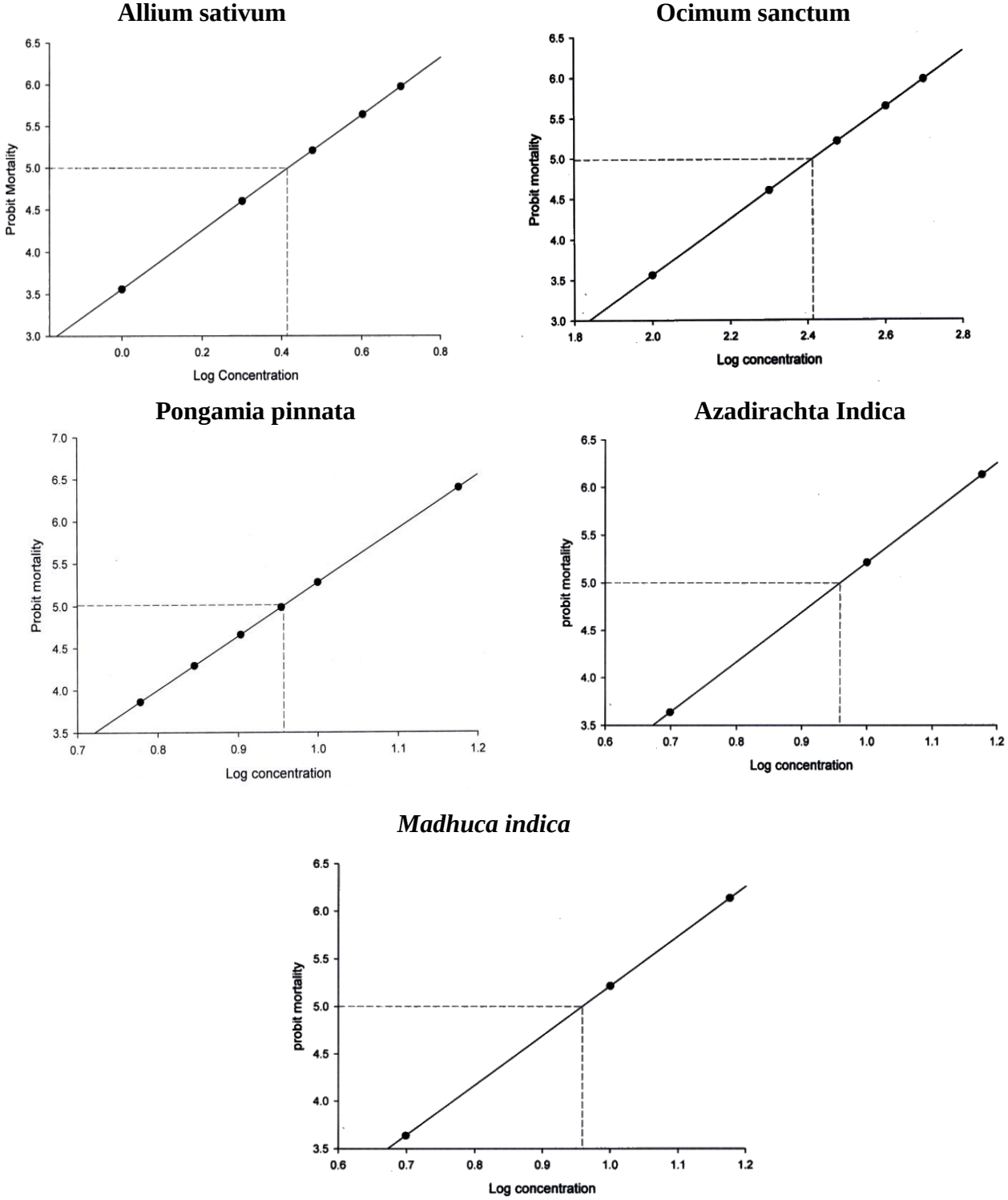

Figure: 1 Probit regression line for plant extracts against larvae of *Aedes aegypti*

Table 2. Larvicidal activity of plant extracts against *Ae.aegypti*

Name of the plant extract	Concentration of test solution in ppm (LC_{50})	Concentration of test solution in ppm (LC_{90})
Allium sativum	3ppm	5ppm
Pongamia pinnata	8ppm	13ppm
Madhuca indica	9ppm	14ppm
Azadirachta indica	10ppm	15ppm
Ocimum sanctum	300ppm	500ppm

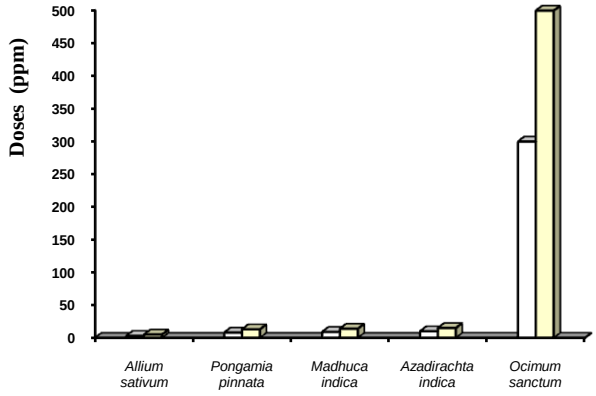

Figure: 2 Graph showing the larvicidal activity of different plant extracts against *Aedes aegypti*

Name of the plant extracts

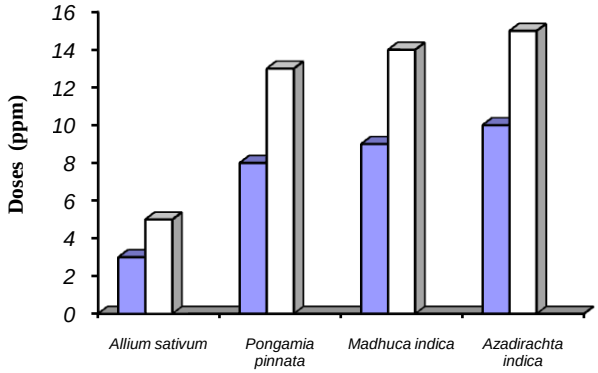

Figure: 3. Graph showing the highest larvicidal activity of different plant extracts against *Aedes aegypti*

The LC_{50} and LC_{90} value of *Ocimum sanctum* (Tulsi leaf extract) against the early IV instar larvae of *Ae.aegypti* was 2.4287 and 2.7972 ppm respectively was shown in table 1.

Table 2 indicates that among the five plant extracts. *Allium sativum* exhibits the highest degree of toxicity against the fourth instar larvae. *Ocimum sanctum* exhibits the lowest degree of toxicity against the fourth instar larvae. The larvicidal effect of plant extracts against the fourth instar larvae decreases in the following order.

Allium sativum > *Pongamia pinnata* > *Madhuca indica* > *Azadirachta indica* > *Ocimum sanctum*.

DISCUSSION

Mosquitoes are a serious threat to public health through which several dangerous diseases are transmitted in both animals and human beings. The cosmo-tropical mosquito *Aedes aegypti* serves as the most important domestic vector of urban yellow fever and dengue. Vector control is a global problem. Management of mosquitoes has been going on over a period of several decades to find a suitable alternative method to replace the chemical control method because of concern about environment pollution and ecological preservation. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. Best strategy has to be integrated with the biological control.

Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. Now a days mosquito control is mostly directed against larvae and only against adult when necessary. This is because the fight against adult is temporary, unsatisfactory and polluting the environment, while larval treatment is more localized in time and space resulting in less-dangerous outcomes. Larval control can be an effective tool to the low mobility of larval mosquitoes, especially where the principal breeding habitats are manmade and can be easily identified.

Recent studies stimulated the investigation of insecticidal properties of plant derived materials or botanicals and concluded that they are environmentally safe, degradable and target specific

botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programmes.

The larvicidal activity of *Allium sativum* against the IV instar larvae was so much effective. It has the larvicidal action even at a very low concentration. Similar observations were reported by Amonkar and Reeves in early 1970 in USA. They reported that the extracted oil and crude methanolic extract of garlic at very low concentrations could control larvae of 5 different mosquito species.

The lethal concentration 50 and 90 value of *Pongamia pinnata* taken for study against the IV instar larvae was 0.8943 and 1.1694ppm, where as crude solvent of leaf, bark and flower extracts of *P.pinnata* showed only moderate larvicidal effects after 24 hours of exposure as was observed by Abdul Rahuman in 2008. He also observed the larvicidal effect of *M. Indica* against *culex quinquefasciatus* which has moderate effect at 1000ppm. Similar observations were made in our study against *Aedes aegypti*. Therefore the *M. indica* exhibit a moderate larvicidal effect on both the species.

In 1995 Ambrose used lower concentration of neemoil on the 3rd and 4th instar larvae of *culex. quinquefasciatus* and showed that they were having LC_{50} values of 0.99ppm and 1.20ppm respectively and for the de-oiled neem cake it was 0.55ppm and 0.72ppm.

The neem seed kernal extracts are effective against mosquitoes were prepared with hexane, ethylether, acetone, ethanol and methane. From this study Tonk *et al.,* in 2006 studied that the most potent larvicide is 0.71% with hexane extract. Okumu *et al.,* 2007 indicated that the larvicidal property of neem oil has higher persistency in 11pm and lasted for 8 days.

In the present study the neem oil extraction process is very simple and easy method. The neem oil is proved to be a larvicide, where the LC_{50} & LC_{90} value was 0.9673ppm and 1.2119ppm. This observation is in par with observation made by above said researchers but the species of mosquito differs.

The toxic effect of *Ocimum Sanctum* extract against the early fourth instar larvae of *Aedes aegypti* was least and this is in par with the observation made by Senthilnathan (2006), where he used many plant extract against fourth instar larvae of *culex quinquefasciatus*.

Thus from our study it reveals that plant extract serves as an effective, alternative, ecofriendly, biodegradable larvicide in controlling the mosquitoes before they disperse and transmit disease.

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