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Larvicidal activity of different natural essential oils against musquito larva

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ABSTRACT

Mosquitoes have been a constant problem in the community they continuously transmit serious diseases. Therefore, this study aimed to eliminate larvae of mosquitoes using different natural essential oils isolated from Neem [azadiractha indica], vitex negundo [nirgundi vitex] and clove [Eugenia caryophyllus]. This was done by comparing the span of time the larvae were exterminated upon exposure to treatments with Five various concentrations of the mixture of essential oils. The time (in minutes) of mortality was measured for each treatment application. Results showed a statistically significant difference between various concentrations of oils with alcohol shows positive control of insecticide and control the alcohol. The various concentrations of oils and ethanol displayed a statistically insignificant difference when compared to the positive control. The different concentrations of essential oils extracts, therefore has larvicidal activity against mosquito larvae, though thorough processing is still necessary for assured efficiency.

Keywords: Larvicidal activity, Mosquito larva, Vitex nigundo oil, Neem oil, Clove oil.

INTRODUCTION

Mosquitoes are one of the most medically significant transmitters as they spread parasites and pathogens, which continue to have devastating effects on human beings. Mosquito-borne diseases such as malaria, filariasis, yellow fever and dengue cause extensive morbidity and mortality and are a major economic burden within disease-endemic countries.

Mosquitoes are very common insects of the family Culicidae. There are about 3,500 different species of mosquitoes throughout the world, of which approximately 230 species can be found in the United States. The three most common mosquitoes found in the United States are *Aedes albopictus, Culex pipens,* and *Anopheles quadrimaculatus.*

Like many insects, mosquitoes go through four life stages that include egg, larva, pupa, and the adult. The female mosquitoes lay their eggs on the water, where they can sit anywhere from 24-36 hours. Once they hatch, the mosquitoes are in their larval phase for 7-14 days. During this time they feed on organic debris and microorganisms in the water while molting their exoskeleton several times until they become pupae.

Eggs

One factor common to all mosquito species is that eggs are laid in association with free water or on a moist surface. Eggs are white when first deposited, darkening to a black or dark brown

within 1224 hours. Single eggs are about 1/50 inch (0.5mm) long, and those of most species appear similar when seen by the naked eye (one exception is the *Anopheles* spp. whose eggs have floats attached to each side of the egg).

Eggs of permanent water mosquitoes where eggs are deposited on the water surface may hatch in 13 days depending on temperature. Floodwater species deposit their eggs on moist oil or another wet substrate and have a wide variation in incubation periods. These eggs will not hatch until submerged by rising water caused by rainfall, melting snow in the spring, or other floodwater. Depending on the species and conditions these eggs may hatch the next time they are flooded, as soon as ten days, or may not hatch until they are flooded a year or more later. *Aedes albopictus* (top), *Culex* spp. (middle), *Anopheles* spp. (bottom).

LARVAE

The larval stage of the mosquito is aquatic. The larvae are legless and spend a majority of time at the surface of the water. The larval stage is commonly referred to as “wriggler” or “wiggler”, due to the lashing movements of the abdomen that move them forward, backward, or sideways in the water.

The larvae (wigglers or wrigglers) of all mosquitoes live in water and have four developmental periods or instars. These are called 1st, 2nd, 3rd, and 4th instars with each succeeding stage larger than the last. At the end of each instar, the larva sheds its skin by a process called molting. The larva is an active feeding stage and must occasionally come to the surface of the water to get oxygen. Some can develop in as little as 5 or 6 days. Upon maturity the 4th instar larvae molts into the pupal stage.

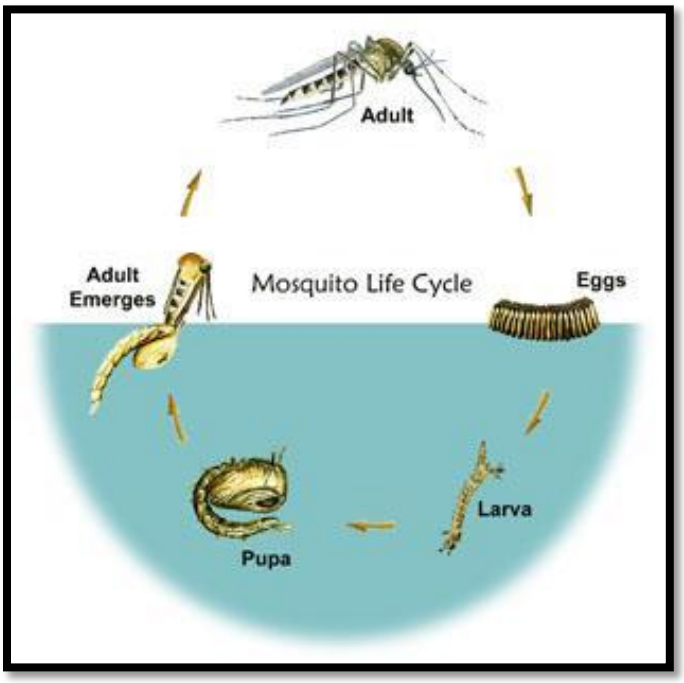
PUPA

Unlike most other insects, the mosquito pupa is very active, and, like the larva, lives in water. It differs greatly from the larva in shape and appearance. The pupa has a comma shaped body divisible into two distinct regions. The front region consists of the head and thorax (cephalothoraxes) and is greatly enlarged. It bears a pair of respiratory trumpets on the upper surface. It must periodically come to the surface to get oxygen. The second region is the abdomen which has freely movable segments with a pair of paddle like appendages at the tip. Feeding does not take place during the pupal stage. The pupal stage only lasts for a few days and is the stage when all the larval tissues change into the adult tissues. The adult emerges directly from the pupal case on the surface of the water.

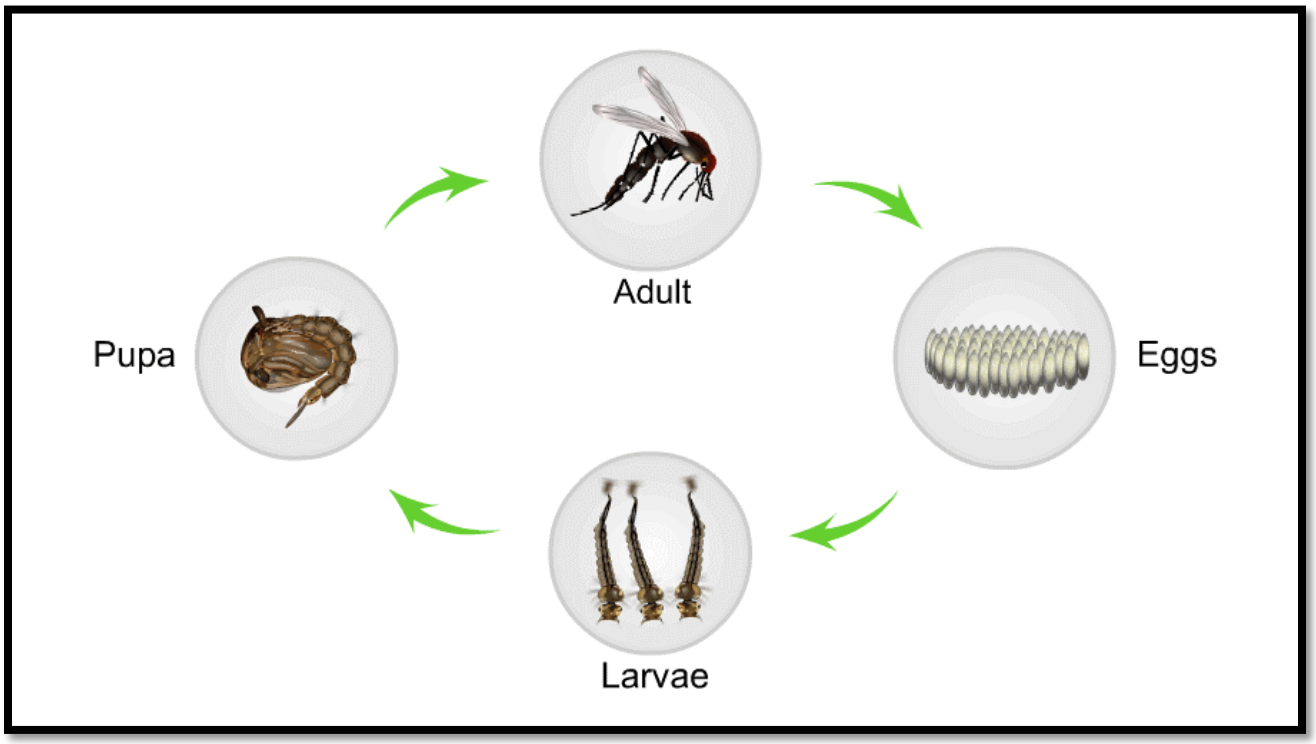
ADULT

The adult mosquito is entirely terrestrial and is capable of flying long distances. Both females and males feed on nectars which they use for energy. Males and females mate during the first 3 to 5 days after they have emerged. Females mate only once. Males generally live for only a week. Only the females feed on blood, which is what is occurring when they are biting. Females evidently gain little nourishment from blood meals but need them in order to develop eggs. Many mosquitoes feed on any warm blooded bird or mammal. However, some prefer coldblooded animals. Some species also prefer birds and seldom feed on mammals, which is the case with *Culex* spp. mosquitoes which are known to transmit the West Nile virus (WNV). Unfortunately many species feed on a wide range of warm blooded mammals and humans are often attacked.

Mosquitoes life cycle



Picture of mosquitoe life cycle.



Stages of Mosquito life cycle

METERIALS AND METHODS

METERIALS: *VITEX NIGUNDO LEAVES, NEEM LEAVES, CLOVE BUDS AND FLOWERS.*

METHODS

Plant material

The leaves of Vitex negundo, Neem [azadiractha indica] and clove buds (eugenia caryophyllus), were collected.

Isolation of volatile oil

The fresh plant materials were collected (1.5 kg) & were subjected to hydro distillation by using a Clevenger-type apparatus for 3 hours according to the method recommended in the British Pharmacopoeia, 1998. The yield of volatile oil obtained was 1.6 % v/w. The collected volatile oil was dried with anhydrous sodium sulphate and stored at 4-6^{0}C in the dark.

GC analysis

The oil from the various herbal drugs was analyzed using a Varian 3300 GC gas chromatograph equipped with a flame ionization detector (FID) ld a DB1 anfused silica column (30 m x 0.25 mm id; film thickness 0.25µm). Injector temperature and detector temperature were 250°C and 300°C respectively. Carrier gas was nitrogen at a linear flow rate of 1.5 ml/min; injector volume for all samples was 0.

STUDY OF MORTALITY OF LARVAE

Preparation of stock solution

One gram of crude extract was first dissolved in100 ml of methanol and stored as stock solution. The anthelmintic assay was carried as per the method of Tandon *et al*. (1997). This stock solution was used to prepare the desired concentrations of the extract for the larvicidal activity on the mosquito larvae. From the stock solution 5, 10, 25, 50, 100 and 200 ppm concentrations were prepared with dechlorinated tap water. The control was set up with 100 ml tap water by adding 2ml of methanol.

Mosquito culture

Culex quinquefasciatus larvae were collected from stagnant sewage water (pits, tanks, and drains). The collected larvae were reared from egg to larval stage and then to adults in the laboratory itself, to avoid the species mixture. From these adults, next F1 generation larvae were used for the present study. This procedure facilitates to maintain the uniform age of
larval stage (fourth instar).

Larvicidal bioassay

Larvicidal activity was evaluated using WHO method (2005) with slight modification. For bioassay test, twenty numbers of early fourth instars larvae were taken in six batches of twenty each for the treatment. Bowls of 100 ml capacity were kept in series, and tested for each desired plant extract concentrations 5, 10, 25, 50, 100 and 200 ppm. The control was set up with 2 ml methanol and distilled water. The experimental media, in which 100% mortality rate of larvae occurred were selected for a dose response bioassay. Based on the screening results, crude methanol solvent extracts of leaf extracts of the plant is subjected to dose response bioassay for larvicidal activity against the larvae of *Cx. quinquefasciatus*. The numbers of dead larvae were counted after every 12 hrs of treatment up to 48 hours. The percentage mortality and standard error of mean have been calculated for all the results obtained by this study.

RESULTS AND DISCUSSION

Table 1. The larvicidal activity of mixture of various plants crude extract at different concentration is represented in Table 1. The data shows that, 100 % mortality rate of mosquito larvae was observed at 25, 50, 100 and 200 ppm of concentrations. And at the concentration of 200 ppm 100 % mortality rate was recorded within 12 hours of the treatment, for 100 ppm it is around 24 hrs, for 50 ppm after 36 hours, and for 25 ppm it was recorded after 48 hrs of the treatment. The efficacy of crude extract on the mosquito larvae showed lesser activity when the concentrations of the same was decreased to 10 and 5 ppm which showed 75 and 45 % mortality rate respectively within 48 hours of the treatment. The control did not show any mortality the mean and standard error also showed in Table 1.

Krishnan *et al*. (2007) [17] reported 50 % mortality rate for the methanolic extract of *Vitex*

negundo leaves at the concentration of 41 ppm and for *V. trifolia* leaves at the concentration of 212 ppm, and Dua et al. (2009) reported 50 % mortality rate for the methanolic extract of *Azardictha indica* (Neem) leaves against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* was found to be 1.6, 1.8 and 1.7 ppm respectively. As the earlier results are in support of the present study the mixture of various plants extract may be considered as the potential control against mosquito larvae which is eco-friendly in nature.

Table 1. Mortality rates of *Culex sp.*mosquito larvae at different concentrations of crude extract of *Vitex negundo* leaves, *Azardictha indica* leaves and *clove* (*eugenia caryophyllus)* buds and flowers.

Conce.	Time (hours)				*n = 20*			
	12	24	36	48	Total	%	Mean	SE (+/-)
5	3	5	7	9	9	45	2.25	1.291
10	7	10	12	15	15	75	3.75	1.683
25	10	14	18	20	20	100	5.00	2.217
50	14	17	20	-	20	100	6.66	1.436
100	18	20	-	-	20	100	10.00	0.500
200	20	-	-	-	20	100	20.00	0.000
Control	0	0	0	0	0	0	0.000	0.000

DISCUSSION

Control of mosquito larvae becomes a very pertinent issue in controlling the rapid replication of mosquitoes in management of vector- borne diseases. In the present study, the mixture of V. nigundo oil, Neem oil and clove oil formulation showed promising larvicidal activity against vectors of various diseases.

Development of resistance in temephos and *Bacillus thuringiensis* is a matter of concern for operational use as larvicides. Although the present formulation may be more costly than other larvicidal agents, such as temephos and *B. thuringiensis*, it has the advantage of being eco friendly, effective and ability to prevent the development of pest resistance.

CONCLUSION

The mixture oils of V. nigundo, Neem and clove were found effective in controlling mosquito larvae in different breeding sites under natural field conditions. The mixture of essential oils from different plants are relative less toxic, eco-friendly and insects are unable to develop resistance and may be used as an alternative to other pesticides for control of vector- borne diseases.

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