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Research Article

SCREENING OF LARVICIDAL ACTIVITY OF COMBINED MEDICINAL EXTRACTS AGAINST DENGUE VECTOR (*AEDES AEGYPTI)*

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ABSTRACT

Mosquitos are vectors for various diseases including Malaria, Dengue, Yellow fever, Filariasis, Japanese encephalitis and Chickungunya. Dengue is the most extensively spread mosquito-borne disease, transmitted by infected mosquitoes of Aedes species. Harmful effects of using synthetic chemical insecticides in mosquito control including vector resistance, environmental pollution and health hazards have initiated the current significance in the search for plant-based insecticide products that are environmentally safe and effective to control mosquito. As a part of the program ethanolic extract of three plant species collected in the state of Tamil Nadu, India were tested for their larvicidal activity against the filarial vector *Aedesaegypti*. The result revealed that the plant extract from the bulb of *Allium sativum,* and leaves of *Andrographispaniculata* and *Rutagravelons* where promising larvicide to control mosquito larvae. Ethanolic extract showed the highest larvicidal activity at the ratio of 1:1:1, 1:4:10, 3:2:1. The result reported in the present study open up the possibility of further investigations on evaluation, identification and isolation of the bioactive components of these plant extracts and its systematic effects on target mosquitoes, which would eventually facilitate the application of the extract as larvicides to control mosquitoes.

INTRODUCTION

In tropical and sub-tropical continent including Asian country, risk of dengue fever is very high. Annually, there are 2 million infections, 500,000 cases of Dengue haemorrhagic fever and 12,000 deaths [1]. Dengue fever was distributed worldwide during 18th and 19th centuries mainly due to the expansion of commerce and shipping industry [2].In India, Mosquito is frequently found due to poor drainage system especially during rainy seasons, fish pond, irrigation ditches and rice fields [3]

Dengue is the most extensively spread mosquito borne disease, transmitted by infected mosquitoes of Aedes species. *Aedesaegypti*is the principal mosquito vector for dengue virus. Dengue fever is basically a viral disease caused by infection with 1 out of 4 serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) of Dengue virus [4]. It is a cosmotropical species that proliferates in water containers in and around houses [5].The bite of *A. aegypti* mosquito transmits the dengue virus in human body. Mosquitoes acquire dengue viruses by feeding on the blood of the infected person. Once a mosquito is infected it gains the capacity of transmitting virus to susceptible individual for its whole life. The infected female mosquitoes may transmit the virus to next generations of mosquitoes through its eggs known as transversal transmission. Humans are main host of virus, where it circulates in the blood of infected person from 2 to 7 days and causes fever at the same time [6]. Dengue fever, or "break bone fever" usually takes 3 to 14 days, (commonly3-7 days) to show severity of high fever after bite of an infected mosquito. Frontal headache, rash, haemorrhagic manifestations, retro-orbital pain, low white blood cell count, nausea and loss of appetite are the basic symptoms of dengue fever. Acute symptoms usually remain for 1 week, but weakness may persist for several weeks [2].

The commercial repellent sprays and mosquito coils use Dithylmetatoluamide (DEET) Methoprene, Briquet, Malathion and Pyrethrum, heavy usage of such chemical agents is proven disadvantages [7]. Most frequently used chemical agents to inhibit the larval population are Temephos and Fenthion [8].Use of synthetic insecticides to control mosquitoes is not well accepted as they are not safe for humans and have damaging effects on environment, non-biodegradable and expensive [7]. Chemical insecticides used to control are harmful for human health as those contains neurotoxic and carcinogenic agents and even carried through food chain which in turn affects the human through bioaccumulation. Moreover due to repetitive use of same insecticide, vectors may become

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resistant [9,10]. Chemical insecticides are also toxic for non-target organisms like bee, butterfly.,etc which have very high ecological importance.

Therefore alternative eco-friendly methods of controlling mosquito larvae are essential for controlling mosquito bearing diseases. Conventionally, plants and their products were used to destroy mosquitoes and other infectious agents. Secondary metabolites of plants possess insecticidal activity (antibacterial, antifungal and larvicidal potential) that protect themselves against herbivorous insects [11].

*Allium sativum*commonly known as garlic, velluli, purunvar and lahsun belongs to a family Amaryllidaceae. It is a perennial herb with a tall, erect flowering stem that grows upto 3 feet. Garlic bulb has been used throughout history for both culinary and medicinal purposes. The species is native to Central Asia, and spread to the Mediterranean region, China and Western Hemisphere[12]. *Andrographispanniculata*commonly known asKalmegh, Bhui-Neem and Kirayat. It is a erect herb extremely bitter in taste which grows upto a height of 30-110 cm in moist shady places. The species is native to India and Srilanka[13]. *Rutagravelons,* commonly called rue, is native to Southern Europe. It is a dicot herb that belongs to Rutaceae family. It is a woody based, Shrubby perennial with aromatic, fern-like, compound leaves which grows in mound to 2-3' tall[14].We prepared the ethanolic extracts of these three plants at various concentrations; ratios and assessed the larvicidal activity against *Aedesaegypti* under laboratory conditions.

MATERIALS AND METHODS

Collection of Plant Sample

The following plants *Allium sativum, Andrographispanniculata, Rutagravelons* were collected from the local areas of in and around Coimbatore district during the month of February 2019. They were collected and kept in sterile bags and then taken to laboratory for further identification by Botanical survey of India (southern circle), Coimbatore.

Processing of Plant sample

The fresh bulbs of *A.sativum,* leaves of *Andrographispanniculata* and *Rutagravelons* were washed with distilled water to remove dirt and dried under shade for 1 week. Then the dried parts were grinded to coarse powder using an electrical blender and then sieved through a mesh to get the fine powders which is stored in sterile airtight container.

Solvent Extraction

8 gram of the each shade dried and coarsely powdered samples were extracted with 90% ethanol individually, in shaker for 48 hours. The extracts were filtered and distilled on water bath, the syrupy mass obtained were dried at room temperature in Petri plates. The dried semisolid ethanolic extracts of each plant were used for the experiments.

Name of the plant	% of yield of extracts
Allium sativum	15%
Andrographispanniculata	30%
Rutagravelons	25%

Collection of Larvae

The larvae of *Aedesaegypti* were obtained from National Center for Disease Control (NCDC), Mettupalayam, Tamilnadu. Larvae were collected in bottles and covered with gauge to avoid entry of any foreign material.

Culture of Larvae

Larvae were maintained at the temperature of 28±2ºC and 80±10% RH (relative humidity) under the 12 hour light and dark photoperiod cycle. The larvae were fed with dog biscuit and a brewers's yeast powder mixture in 3:1 ratio whuch is used in the laboratory [15].

Larvicidal Bioassay

Larvicidal activity was evaluated in accordance with WHO standard with slight modification being done for study [16].1 gram crude extract of each plant were made upto 10 ml with ethanol and kept in 3 separate beakers. From each beaker 3 ml of each extract alone was taken and poured in containers labelled 1, 2 and 3 containing 3 ml different plant extract and 247 ml water. Then different concentration of extracts were taken and mixed well which gives 3 ml. Likewise 6 different compositions labelled 4-9 were made with 3 different extracts individually, filled with 3 ml of extract composition (4-9) and 247 ml water. The 10^{th} container labelled is Control, filled with 250 ml water only. Larvae in batches of 15 were carefully transferred in all the 10 containers with the help of dropper. The larvicidal bioassay was performed at room temperature. The beakers were covered with muslin cloth after the insertion of larvae into the beaker, to avoid contact with the foreign particles. After exposure the larvae in the beakers were observed and outright recorded. In between the experiment, no food was ceded to larvae. At the end of 48 hrs, the observed mortality was recorded. Ther is no sign of any movement even after a mild touch with a glass rod [17]and dead larvae are to be counted as described in the WHO technique report series Percentage of mortality of larvae in each container was observed and overall mortality rate was calculated after 48 hrs using the formula

Percentage mortality = Number of dead larvae/ Number of larvae introduced * 100

RESULTS

Literatures are full of scientific documentation regarding medicinal plants and they have a potential to cure various diseases [18]. Thus this future further encourage to manufacture a biopesticidal products synthesized from plants as they are safe as compared to synthetic pesticides, which are not only costly and have adverse effects too [19].

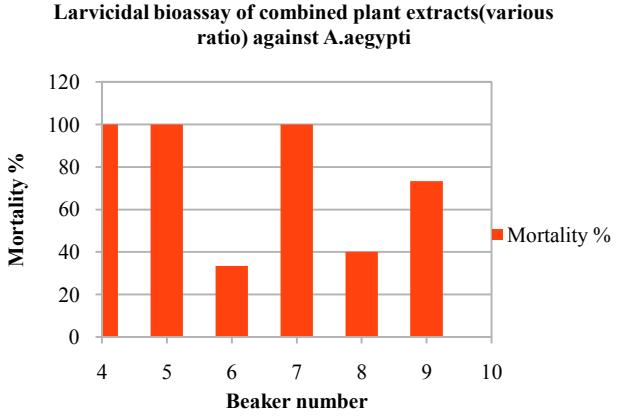
Mortality of *A.aegypti* after the treatment of different types of concentrations of plants namely *A.sativum, A.paniculata*and *R.gravelons* are shown in **Table 1,2.**

Table 1 Larvicidal bioassay of individual plant extracts against *A.aegypti* (out of 15)

Beaker.No	Plant extract	No of Live larvae	No of Dead Larvae
1	*A.sativum*	9	6
2	*A.paniculata*	11	4
3	*R.gravelons*	12	3

Table 2 Larvicidal bioassay of combined plant extracts(various ratio) against *A.aegypti* (out of 15)

Beaker. No	Extract ratio *A.sativum* : *A.paniculata* : *R.gravelons*	No.Live Larvae	No. Dead Larvae	Mortality (%)
4	1 : 1 : 1	-	15	100%
5	1 : 4 : 10	-	15	100%
6	1 : 10 : 4	10	5	33.4%
7	3 : 2 : 1	-	15	100%
8	1 : 2 : 2	9	6	40%
9	1 : 3 : 3	4	11	73.3%
10	CONTROL	15	0	0%



DISCUSSION

Mosquito life cycle follows eggs, larval stage, pupa and adult stage. Majority of the mosquito control programs target on the larval stage in their breeding sites [20,21].The larval stage is more attractive target to kill them as it moves on water at this stage, which makes it easy to deal with them in its own habit. Some fishes also take this larvae as their foods. The use of conventional chemical insecticides in the water sources, ultimately introduces many health risks to people and our ecosystem. Insecticides manufactured from plants are more acceptable in this regard.

In this study, the mortality percentage of larvae after 48 hrs of observation after the treatment of individual extracts and different combinations of extracts were tested against larvae of *A.aegypti.* The result indicates that combined formulations of *A.sativum, A.paniculata* and *R.gravelons* exerted an interesting larvicidal activity when compared to effect of individual extracts. The combinations where the extracts in the ratio of 1:1:1, 1:4:10, 3:2:1 were showed better larvicidal activity of 100% mortality, when compared to other combinations. The investigation provided a platform and scope for the use of these plants at these combinations as potential larvicidal agent in control of dengue larvae (*A.aegypti*larvae)

CONCLUSION

The present strategy might be used to control dengue vector. This approach could reduce the possibilities of physiological resistance development in mosquito population. Before that, the mode of action and larvicidal efficiency of different combinations of the plants extract under field conditions should be evaluated. Besides further investigation is required regarding the effect on non-target organisms, which is extremely important.

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Conflict of Interest: Nil

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