SCREENING OF LOCAL PLANT SPECIES FOR LARVICIDAL ACTIVITY

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Abstract

Mosquitoes have become one of the major public health problems all around the world. Control measures currently used are directed against only one or some of the most important species and aimed at the adults or the larvae. Mosquito control is a difficult task and has become more difficult; because of variety of factors including the development of insecticide resistance and worry over environmental pollution and many of them are immune-suppressants. Environmental protection agencies have banned or placed severe obligations on the use of many pesticides which were formerly used in mosquito control programs. One possible strategy is the rational localization of bioactive products from phytochemicals by precisely exploring the global floral biodiversity. Biologically active plant materials have attracted considerable relevance in mosquito control catalogue in the modern times. The present study deals with the screening of locally available plants for mosquito larvicidal properties such as Argemone mexicana L., Cassia glauca Lamk., Dendrocalamus strictus Nees., Leucas martinicensis (Jacq.) R. Br., Pongamia pinnata(L.) Pierre and Solanum xanthocarpum Schard & Wendl.

Key Words*: Mosquito larvae, phytochemicals, biologically active plant materials, larvicidal, etc.*

INTRODUCTION:

Mosquitoes have become major public health pests throughout the world. Out of 3000 species of mosquitoes recorded worldwide nearly, more than hundred species are capable of transmitting various diseases to human. Mosquitoes transmit many pathogens and parasites such as bacteria, protozoan, viruses and nematodes (Das & Ansari, 2003). Mosquitoes are the major vector for transmitting malaria, dengue fever, yellow fever, filariasis, schistomiasis and Japanese encephalitis. In India, malaria is one of the most significant causeof direct or indirect infant, child and adult death with approximately two to three million new cases emerging every year. *Anopheles subpictus* Grassi is spread throughout India, Afghanistan, Borneo, China, Malaysia, Philippines, Sri Lanka, Java, Bangladesh and Indonesia. It is a dominant species in Haryana as well as Uttaranchal states. Though it is not a vector species, same infected specimens with malarial parasite are reported from India, Indonesia and Java (Kamraj C.*et al*., 2010).

World Health Organization (WHO) stated that about 2 out of 5 of the global human population are currently threatened of dengue and the best way to control the conveyance of dengue virus is to destroy the mosquitoes that cause the disease (Pedro J. *et al*, 2014). Dengue fever has emerged as a serious public health problem in the world, mainly in tropical countries where the amicable environmental conditions are responsible for the mutiplication of vectors *Aedes aegypti*. Amongst the arbovirus in India, dispensed of all the dengue virus typeshas continuously expanded (Rajasekaran and Duraikannan, 2012).

Control measures are generally used against only one or a few of the most important species and can be aimed at the adults or the larvae. Mosquito control is a difficult task and is becoming even more, due to a variety of factors including the development of insecticide resistance and concern over environmental pollution and many of them are immune-suppressants. Because of resistance in the vectors, conventionally insecticides, the chief weapon of the vector, are becoming ineffective. Mosquitoes are also becoming increasingly resistant to traditional chemical pesticides and there is growing concern about the existent health and environmental risks surrounding these grave products.Environmental protection agencies have prohibited or placed severe restrictions on the use of many

pesticides which were formerly utilized in mosquito control programs and there are now fewer adulticides available than for last 20 years (Kamaraj *et al*., 2011). Presently organophosphate, organochloride and synthetic pyrethroid insecticides are used for public health control mensurates. Consecutive changes in insecticides are results in multiple insecticide resistance. Malaria vectors in India have become resistant to DDT, Malathion and Deltamethrin (Patil *et al*., 2010). This phenomenon has actuatedand urged the development of optional techniques using natural products. Current research trends use plant extracts as substitutelarvicides because they contain varied phytochemicals that are specific in killing mosquito larvae without affecting other organisms and the environment. Instead of using synthetic larvicides, the use of these plant-obtained products in controlling mosquito larvae is cheap and ecofriendly (Pedro J. *et al*., 2014).

One possible technique is the rational localization of bioactive products from phytochemicals by systematically exploring the global biodiversity of flora. There is an ever-increasing demand for plant-based insecticides as they are nontoxic, easily available at cheap prices, biodegradable and show broad spectrum target specific activities against different species of vectors. Furthermore, unlike conventional commercial insecticides that are based on single active ingredient, plant derived insecticides that are based on single active ingredient, plant derived insecticides comprise blends of secondary metabolites which act on both behavioral and physiological process (Bagavan and Rahuman, 2011). Many of the herbs and shrubs are found to have promising medicinal properties, mosquito larvicidal and mosquito repellent properties (Nath *et al*., 2006). The plant world consists a rich storehouse of phytochemicals, which are extensively used in the place of chemical insecticides since continuous use of synthetic insecticides cause side effects to non-target or beneficial organisms and insecticide opposition of mosquitoes. Phytoproductshave minimal hazardous effect on the environment and wide present over a range can promise, in future for mosquito control programs. They had revolutionized the fields of vector restrain as they possess different bioactive compounds and can be used as general toxics against the larval stages of the mosquito (Arivoli *et al*., 2012)

Insecticides derived from plants have been extensively used on agricultural pests, but to a very limited extent, against insect vectors of public health benefit, which demand careful and rigourous screening. Biologically active plant compounds have earned considerable interest in mosquito controlling programs in the present times. The current study comprises of the screening of locally available herbs and shrubs for mosquito larvicidal properties (Nath *et al*., 2006).

MATERIAL AND METHODS:

Plants used for the study were *Argemone mexicana* L., *Cassia glauca* Lamk., *Dendrocalamus strictus* Nees., *Leucas martinicensis* (Jacq.) R. Br., *Pongamia pinnata*(L.) Pierre and *Solanumxanthocarpum* Schard & Wendl.

Collection of plant material:

A total of six plants belonging to diverse families and genera were collected from locality of Kapsilake, Akola. Collected plants were identified by Prof. Dr. S. P. Rothe, Head, Dept. of Botany, Shri Shivaji College, Akola. The list of plants is mentioned in Table 1.

Table 1: List of Plants selected for study.

Sr. No.	Name of Plant	Family	Locality of Collection
1	*Argemone mexicana* L.	Papaveraceae	Kapsi Lake, Akola
2	*Cassia glauca* Lamk.	Ceasalpiniaceae	
3	*Dendrocalamus strictus* Nees.	Poaceae	
4	*Leucas martinicensis* (Jacq.) R. Br.	Lamiaceae	
5	*Pongamia pinnata*(L.) Pierre	Fabaceae	
6	*Solanum xanthocarpum* Schard & Wendl.	Solanaceae	

Preparation of plant samples:

Plants collected from different families were brought to the laboratory, washed with dechlorinated water, shade dried under room temperature for about 7-15 days and the plant leaves were powdered individually using an electric blender. The blended powder of each plant was sieved by strainer.

Preparation of plant extracts:

10 gm of plant powder was weighed and extracted with particular solvent and allowed to stand for about 48 hours with stirring at regular intervals. After 48 hours the plant extract was filtered with Whatman filter paper No. 1. This

filtered extract was allowed to dry in petri dishes in oven at 40^0 - 45^0C. Further the dried matter was weighed and 1gm was dissolved in 10 ml of respective solvent and stored in bottles. (Table 2).

Table 2: Organic plant extract preparation using various solvents.

Sr. No.	Name of Plant	Solvent used for extract
1	*Argemone mexicana* L.	Methanol
2	*Cassia glauca* Lamk.	Hexane
3	*Dendrocalamus strictus* Nees.	Petroleum ether
4	*Leucas martinicensis* (Jacq.) R. Br.	Methanol
5	*Pongamia pinnata*(L.) Pierre	Ethanol
6	*Solanum xanthocarpum* Schard & Wendl.	Methanol

Collection of Mosquito larvae:

Mosquito larvae were collected from an open tank near girls hostel of Shri Shivaji College, Akola. Each time fresh larvae were collected for bioassay. The larvae were kept at 25^0 – 27^0 C. As the study is aimed only for screening of larvicidal activity of plants, identification of larval species was not done.

Larvicidal Bioassay:

Plant extract bioassay for larvicidal activity was performed using 10 larvae per 100ml dechlorinated tap water. For each plant extract 5 beakers were used with extract concentration 0 ml (Control), 0.1 ml, 0.2ml, 0.5ml and 1 ml. After adding plant extract beakers were allowed to stand for 24 hours. The other day, number of dead larvae was noted down and percent larval mortality was calculated using formula:

$$\% \text{ Observed Mortality (CM)} = \frac{Total\ No.of\ dead\ larvae}{Total\ no.of\ larvae}\ x\,100$$

If the control mortality is between 5% and 20% the percentage mortalities should be corrected by Abbot's formula

$$\% \text{ Mortality(M)} = \frac{\% \text{ of test mortality} - \% \text{ of control mortality}}{100 - \% \text{ ControlMortality}} \text{x } 100$$

If control mortality is below 5% it can be ignored and no correction is necessary.

OBSERVATIONS AND RESULTS:

Table 3: Larvicidal activity of *Argemone mexicana* L. plant extract.

Amount of extract used (ml)	Working concentration of extract (mg/ml)	Methanol (ml)	No. of larvae before incubation	No. of dead larvae after incubation	% larvae mortality
00	00	1.0	50	10	20
0.1	10	0.9	50	25	50
0.2	20	0.8	50	35	70
0.5	50	0.5	50	40	80
1.0	100	00	50	50	100

Table 4: Larvicidal activity of *Cassia glauca* Lamk. plant extract.

Amount of extract used (ml)	Working concentration of extract (mg/ml)	Methanol (ml)	No. of larvae before incubation	No. of dead larvae after incubation	% larvae mortality
00	00	1.0	50	15	30
0.1	10	0.9	50	20	40
0.2	20	0.8	50	30	60
0.5	50	0.5	50	44	88

1.0	100	00	50	50	100

Table 5: Larvicidal activity of *Dendrocalamus strictus* Nees. plant extract.

Amount of extract used (ml)	Working concentration of extract (mg/ml)	Methanol (ml)	No. of larvae before incubation	No. of dead larvae after incubation	% larvae mortality
00	00	1.0	50	15	30
0.1	10	0.9	50	18	36
0.2	20	0.8	50	22	44
0.5	50	0.5	50	28	56
1.0	100	00	50	33	66

Table 6: Larvicidal activity of *Leucas martinicensis* (Jacq.) R. Br. plant extract.

Amount of extract used (ml)	Working concentration of extract (mg/ml)	Methanol (ml)	No. of larvae before incubation	No. of dead larvae after incubation	% larvae mortality
00	00	1.0	50	10	20
0.1	10	0.9	50	12	24
0.2	20	0.8	50	22	44
0.5	50	0.5	50	25	50
1.0	100	00	50	32	64

Table 7: Larvicidal activity of *Pongamia pinnata* (L.) Pierre plant extract.

Amount of extract used (ml)	Working concentration of extract (mg/ml)	Methanol (ml)	No. of larvae before incubation	No. of dead larvae after incubation	% larvae mortality
00	00	1.0	50	20	40
0.1	10	0.9	50	20	40
0.2	20	0.8	50	25	50
0.5	50	0.5	50	32	64
1.0	100	00	50	46	92

Table 8: Larvicidal activity of *Solanum xanthocarpum* Schard & Wendl. plant extract.

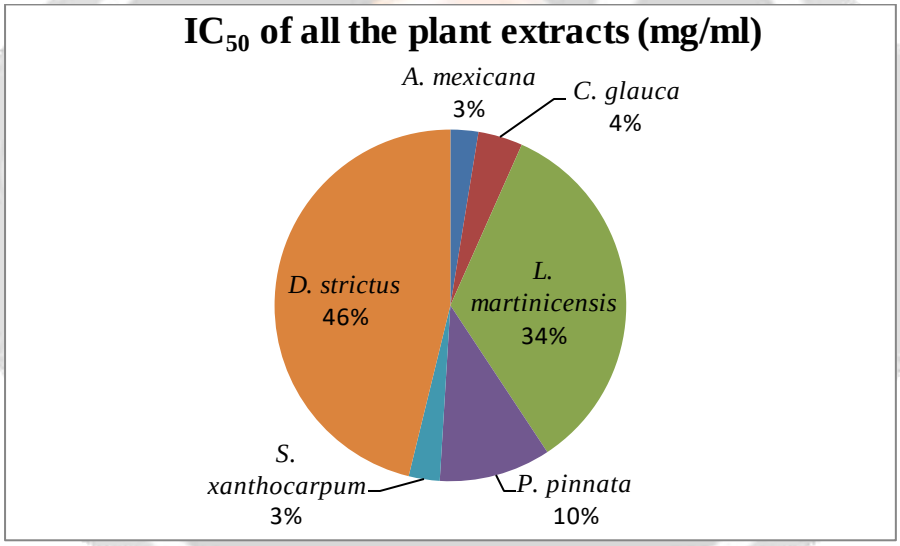
Amount of extract used (ml)	Working concentration of extract (mg/ml)	Methanol (ml)	No. of larvae before incubation	No. of dead larvae after incubation	% larvae mortality
00	00	1.0	50	10	20
0.1	10	0.9	50	20	40
0.2	20	0.8	50	26	52

0.5	50	0.5	50	36	72
1.0	100	00	50	42	84

The larvicidal effect of various plant extracts was evaluated in dose-dependent manner and the efficacy of the plant extracts was expressed as IC_{50} value i.e. inhibitory concentration of the plant extract required to induce 50% larval mortality following the treatments. The concentrations of the extracts ranged between 10-100 mg/ml of extract; of the total six plant extract tested, the 100% larvicidal activity was reported for *Argemone mexicana* L. plant extract. However, the lowest IC50 was reported for *Argemone mexicana* L. plant followed by *Solanum xanthocarpum* Schard & Wendl. and *Cassia glauca* Lamk. respectively. (Table 9).

Table 9: IC_{50} of all the plant species studied.

IC_{50} of all the plant extracts (mg/ml)					
***A. mexicana* L.**	*C. glauca* Lamk.	*D. strictus* Nees.	*L. martinicensis* (Jacq.) R. Br.	*P. pinnata* (L.) Pierre	*S. xanthocarpum* Schard & Wendl
14.89	23.77	268.4	197.9	59.64	16.57



Argemone mexicana L. and *Solanum xanthocarpum* Schard & Wendl. are found to give promising results and this larvicidal activity is due to the secondary metabolites present in them. Different types of secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, lignins, saponins, sterols and tannins were present. Current reports show that tannins may have potential value such as cytotoxicity and antineoplastic agents. Saponin have the anti-fungal properties. These contents show different types of activities against different pathogens. Therefore, it can be used in the treatment of disease.

CONCLUSION:

Mosquitos are the major vectors for the transmission of malaria, dengue fever, filariaisis, schistosomiasis, yellow fever and Japanese encephalitis (Das and Ansari, 2003). In India, malaria has become one of the most important cause of direct or indirect infant, child and adult death with approximately 2-3 million new cases arising every year. Due to pathogenic diseases and lethal harms caused by the mosquitoes, controlling them has been the primary subject of several new researchers over the past few years (Invest and Lucas, 2008). Instead of using synthetic larvicides, the leaves of these plant derived products in controlling the mosquito larvae is inexpensive and environment friendly. In the present research work it has been evaluated the larvicidal effect of the local medicinal plant species against mosquito larvae. It is found that plant extract of *Argemone mexicana* L. has shown potent

larvicidal activity followed by *Solanum xanthocarpum* Schard & Wendl. and *Cassia glauca* Lamk.. Thus, these plants have significant larvicidal potential that can be exploited against mosquito control.

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